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JOURNAL OF DAIRY SCIENCE

VOLUME XVII

JANUARY, 1934

NUMBER 1

EFFECT OF CALCIUM-DEFICIENT ROUGHAGES UPON MILK YIELD AND BONE STRENGTH IN CATTLE

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INTRODUCTION

Dairy cows depend upon roughages as the principal natural sources of calcium, and upon grains and milling by-products for most of the phosphorus. Grasses provide from one-fifth to one-third as much calcium, and about three-fifths as much phosphorus as do the legumes grown under the same conditions. The calcium content of the forages grown on low-lime soils is lower than that of the same kinds of plants on more fertile soils. Lactating cows dependent on grass forages grown on low-lime soils often deplete their reserves of calcium to such an extent that skeletal strength and yield of milk are affected.

STATEMENT OF PROBLEM

The Jersey cows in the Florida Agricultural Experiment Station dairy herd were dependent upon pasture grasses, and corn or sorghum silages, as the main sources of roughages. Since December, 1920, alfalfa meal was included as one-ninth to one-eighth of the concentrates mentioned in the herd records and annual reports of the station. One cow—No. 18—received alfalfa meal prior to that time, while she was on Register of Merit test.

All rations mentioned during this time contained an adequate proportion of protein, total digestible nutrients and phosphorus for cows of their weight and milk production. Under the conditions of feeding just mentioned, these Jerseys had an average milk yield of approximately 4,000 pounds per year. A number of them suffered broken bones—hips, ribs and even a pelvis—under this general feeding practice.

The rations were changed in January, 1929, by the addition of two per cent of bonemeal to the mixed concentrates, based upon a study of the conditions noted. Bonemeal was chosen in preference to a local supply of calcium carbonate because the former was known to be free from fluorine and other undesirable substances. This proportion of mineral supplement

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was assumed to supply sufficient calcium to provide for maintenance, a reasonable milk production, and to allow a surplus with which the cows could restore the body reserves.

REVIEW OF LITERATURE

Eckles (2) observed that cows on phosphorus-deficient rations produced much less milk than when the lacking mineral was supplied. Theiler (7) and Tuff (8) reported similar conditions among cattle in parts of the Union of South Africa and of Norway. Fingerling (3) obtained similar results with milk goats on rations the basal portion of which was deficient in calcium. Meigs and Woodward (5) concluded that cows produced appreciably more milk after having been placed in good mineral storage during the preceding dry period.

Kellner (4) mentioned that dairy rations are deficient in calcium more frequently than they are in phosphorus. Under these conditions, cows withdraw mineral matter from the bones in order to maintain milk production. As this impoverishment progresses, the milk yield is decreased.

Nessler (6) found that the bones of a cow affected with *Knochenbruchigkeit* (bone-fragility) contained less calcium and phosphorus, were lighter in weight, and had thinner shaft walls than had those of a healthy cow. The spongy bones—vertebrae, pelvis and joints—were affected more than the shaft bones of the legs. This depletion of calcium and phosphorus in the skeleton occurred when the cattle received feeds inadequate in the bone-forming mineral elements.

PLAN OF INVESTIGATION

Typical rations used with the dairy herd were assembled from the herd records and annual station reports, and a study made of them. The rations were changed slightly in January, 1929, by adding two per cent of finely ground feeding bonemeal to the concentrates. Some of the higher producing cows were given a daily allowance of about five pounds of alfalfa hay. Some changes were made in the proportion of concentrates based on local market prices of nutrients, and upon availability of these feeds.

Twelve Jersey cows in the herd had completed lactations on the unsupplemented rations, and also upon the supplemented rations after January, 1929. Their lactation records were assembled by 10-day periods, computed to maximum age basis, using the factors of Clark (1) for Jersey cows milked twice daily not under official test conditions. An average lactation curve was computed, weighted on the "per cow" basis.

Cow No. 59 broke her pelvis late in December, 1928. Following the autopsy, breaking strengths were determined upon her femurs and humeri, using an electrically driven Riehle Brothers testing machine, and applying the weight slowly from above in the middle of 7- and 5-inch spans of the

shafts. After bonemeal had been added to the rations, leg bones were obtained from additional Jersey cows as they were eliminated from the station herd, and bone strengths determined similarly, using a 6-inch span. Similar data were obtained from the leg bones of three range cows, six Aberdeen Angus cows and 24 three-year old steers. The steers had free access to bonemeal during one year prior to slaughter, while the Angus cows received a limited grain allowance which contained one per cent of bonemeal. Both the steers and Angus cows received pasture grasses and silages grown on the same types of soil as that fed to the Jerseys.

PRESENTATION OF DATA

The data presented herein were accumulated under the conditions of management prevailing over a period of years in the station dairy herd generally, rather than from an investigation planned in advance with a limited number of selected cows. Detailed search of the herd records and experiment station reports showed that pasture grasses (largely Bahia, centipede and Bermuda grasses), corn, cane or sorghum silages were the principal roughages in use. Summation of published data from seven early feeding trials between 1908 and 1917 showed that Jersey cows with an average weight of 711 pounds and a 15 pound milk yield daily, received 22½ pounds of silage. Their concentrates were mainly one part of cottonseed meal (36% protein) with 2 to 4.4 parts of wheat bran. Other concentrates fed at times included cocoanut meal, wheat middlings, velvet beans in the pods or ground into feed meal. One test cow ration consisted of 300 wheat bran, 200 velvet bean feed meal, 100 cornmeal and 80 parts of peanut meal (grade not stated). Alfalfa meal was mentioned first in the ration of Cow No. 18 on Register of Merit test, and again in December, 1920, when it became one-ninth to one-eighth of the regular concentrate mixtures fed. During two feeding trials of 123 and 84 days, 4 and 6 cows respectively received not in excess of four pounds of alfalfa hay daily. Mineral supplements, except common salt, were not used until shortly prior to 1929, and then for but a limited time.

The concentrate mixtures used more recently in connection with the roughages mentioned are listed in table 1. Cost and availability of oats, corn gluten feed and peanut meal (44% protein) caused them to be replaced with locally grown velvet bean feed meal, higher grade cottonseed meal and by changing the proportions of the feeds used.

Calcium and phosphorus contents of the several typical rations were calculated, using average analyses of purchased concentrates, and analyses of the locally-grown feeds. All of these rations provided an adequate supply of protein and total digestible nutrients, and exceeded the requirements given by Kellner (4) and Wellman (9) for phosphorus. The supply of calcium was noticeably inadequate.

TABLE 1

Concentrates used with silages and grass pasture in feeding the Florida Station dairy herd from 1922 to 1933

YEAR	1922	1923	1924 AND 1925	1928	1929 TO 1933
Ration	Ration 1	Ration 2	Ration 3	Ration 4	Ration 5
Wheat bran	100	100	100	200	400
Cornmeal	100	100	100	300	300
Ground oats	75	75	100	300	
Alfalfa meal	50	50	50		
Peanut meal	50			100	
Cottonseed meal, 36%	50	100	100	100	
Cottonseed meal, 41%					100
Corn gluten feed				100	
Dried beet pulp				100	200
Linseed oilmeal				100	100
Velvet bean feed meal					200
Common salt					13
Bonemeal					26

The concentrates in general use since January, 1929, containing two per cent of bonemeal, provided calcium sufficient to meet the requirements for maintenance, a more liberal milk yield, and to allow mineral reserves to be restored to the skeleton. No cow in milk in the station herd has had a broken bone since that time, and the milk yield has increased, as will be shown. A comparison of the amounts of calcium and phosphorus contained in these several rations is shown in table 2.

Lactation records were assembled and tabulated for the 12 Jersey cows that had completed lactations both on the earlier low-calcium rations with the concentrates listed in table 1, and on the rations containing bonemeal. A total of 44 lactations were included in the low-calcium group. The average actual yield per lactation, weighted on the "per cow" basis, was 3,980 pounds of milk, or an average of 13.38 pounds daily during these 44 lactations of 400 days or less. These same 12 cows completed 22 lactations on the high-calcium rations, averaging 6,425 pounds of milk, or 17.55 pounds daily. The earlier group of lactations averaged 297 days in length, in contrast to 366 days for the latter group.

There was a marked tendency for these cows to attain a higher maximum daily milk yield, and to decline in milk flow less rapidly when the low-calcium rations had been supplemented with bonemeal.

Since these lactations were begun at varying ages, they have been computed to a uniform maximum age basis, using the factors obtained by Clark (1) with Jersey cows milked twice daily not on official test. The lactation curves so computed, are shown in figure 1. These milk records

TABLE 2

Comparison of the nutrients required by a typical cow in the station dairy herd and of the nutrients provided by rations in use between 1908 and 1933

	RATIONS		NUTRIENTS PROVIDED			
	Corn silage	Concentrates	Digestible crude protein	Total digestible nutrients	Calcium (Ca)	Phosphorus (P)
	pounds	pounds	pounds	pounds	pounds	pounds
Requirements— 725 lb. cow yielding 15 lbs. of 5% milk*			1.5050	11.4756	0.0800–.1059 .0789***	0.0394–.0458** .0323***
Rations in use—						
1908–1917	22.25	12.0	2.3178	11.6633	.0306	.1705
1922–1925	22.25	11.0	1.907	11.638	.0448	.1973
1928	22.25	10.5	1.8733	11.6600	.0408	.1535
1929–1933	22.25	11.0	1.6811	11.6075	.0776	.1032

*—The average requirements are based on weights and milk records of cows in feeding trials conducted between 1908 and 1917 at this station.

**—Calcium and phosphorus requirements calculated according to Wellman's (9) requirements.

***—Calcium and phosphorus requirements calculated according to Kellner's (4) requirements.

were assembled by 30-day periods, and the relative rates of milk yields calculated in relation to the milk yield on the unsupplemented rations. This

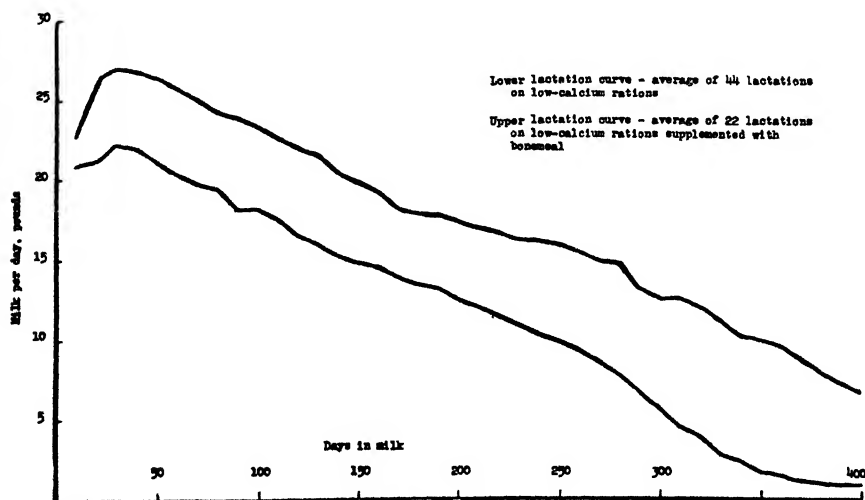


FIG. 1. Average daily milk yields of 12 Jersey cows before and during use of bone-meal as a supplement to low-calcium rations.

comparison of monthly milk yields on the low-calcium and on the supplemented rations is shown in table 3.

TABLE 3

Persistence of milk production of 12 Jersey cows as affected by addition of bonemeal as a supplement to low-calcium rations

MONTH	PRODUCTION ON LOW-CALCIUM RATIONS		PRODUCTION ON SUPPLEMENTED RATIONS	
	Milk yield	Rate of production*	Milk yield	Rate of base production*
	<i>pounds</i>	<i>per cent</i>	<i>pounds</i>	<i>per cent</i>
1	644.3	13.84	765.1	16.43
2	636.2	13.66	789.9	16.96
3	572.1	12.29	732.1	15.72
4	525.5	11.29	681.3	14.63
5	464.0	9.96	622.2	13.36
6	420.6	9.03	553.2	11.88
7	378.2	8.12	525.3	11.28
8	328.2	7.05	493.7	10.60
9	281.2	6.04	464.1	9.97
10	201.9	4.34	406.7	8.73
11	112.6	2.42	359.0	7.71
12	55.4	1.19	295.4	6.34
13	27.6	.59	237.7	5.11
10 days	8.4	.18	66.2	1.42
Total	4,656.2	100.00	6,991.9	150.16

*—The total production in the first lactation curve (4,656.2 pounds of milk) is the base figure used in computing the rate of production. These lactation curves are weighted on the "per cow" basis, computed to the maximum age by using the factors of Clark (1), so as to place individual lactations upon a comparable basis.

Over fifty per cent more milk was produced per lactation of 400 days or less on the supplemented rations. These 12 cows yielded 31.17 per cent more milk per day for each day in milk (under 400 days) during these lactations. This latter point takes into consideration the fact that the cows tended to milk over longer lactation periods on the supplemented rations.

SKELETAL STUDIES

Five cows in a herd of 34 on the low calcium diet had suffered one or more broken bones, as follows:

- No. 59—pelvis broken in three places.
- No. 120—both hips and right 12th rib broken.
- No. 223—right 13th rib.
- No. 225—left hip and last four ribs on right side.
- No. 229—left hip.

Even a 10-year old bull—Florida's Majesty 153431—was slaughtered in 1927 because of a broken hip that affected his usefulness. This large pro-

portion of animals with broken bones called attention to the extremity of the condition caused by the low-calcium rations. Cow No. 59 went down with a broken pelvis during late December, 1928. Upon autopsy, the femurs and humeri were obtained, and average breaking strengths of only 335 pounds were recorded. In these determinations, 7- and 5-inch spans respectively were used, but in all subsequent studies of bone strengths, a standard 6-inch span was adopted. As more cows were eliminated from the herd, the long bones of the legs were obtained, and their strengths determined.

It is of interest to note that the femurs and humeri of four Jersey cows receiving the supplemented ration for the last 19 to 27 months, averaging over 10 times as strong as the corresponding bones taken from Cow No. 59. In every instance the femurs and humeri exceeded the cannon bones in average strength. Noting this relationship, it was possible to use strengths of cannon bones as indices of the state of mineral storage of the cows.

The strengths of No. 59's leg bones represent an extreme stage of depletion of mineral reserves in the skeleton. This cow had been a persistent producer, averaging 6,338.6 pounds of milk in her 11 lactations, and in the last lactation produced 8,151.9 pounds of milk in 531 days. That this cow is not an isolated instance is suggested by the proportion of cows in the station herd at that time that had broken one or more bones.

The strengths of cannon bones from 24 three-year old steers represent a medium stage of mineral storage in the skeletal tissues. Three mature (dry) range cows for which bone strengths are presented, were in a lower stage of storage. On the other hand, the bone strengths of the dairy cows that had received the supplemented rations indicate that it may be possible for cows to attain a stage of excellent mineral storage, available for subsequent lactations. A summary of these bone strengths, as determined on selected bones taken from cattle under the various conditions outlined, are presented in table 4.

DISCUSSION OF RESULTS

The cows considered in this study received rations that were adequate, considered from the usual feeding standards. This was evidenced by the fact that many of the cows were extremely fat, and yet were not yielding milk in proportion to the intake of digestible nutrients—in many instances they had one pound or more of grain for each two pounds of milk produced. The quality of protein was probably satisfactory, since it was derived from six plant sources—maize, wheat, flax, velvet beans, cotton and sugar beets—in addition to the variety of pasture grasses. The supply of phosphorus from the concentrates was in excess of the requirements. The calcium intake in the average ration was extremely low, even for cows with the level of milk production of Jerseys in commercial dairies.

The depression of milk production was less marked than has been observed in long-continued phosphorus deficiency (2).

TABLE 4
Average breaking strength of 215 leg bones from Florida cattle under different feeding conditions*

CATTLE	TYPE OF RATION	AVERAGE BREAKING STRENGTH OF BONES FROM LEFT AND RIGHT LEGS						
		Humerus	Femur	Radius and ulna	Tibia and fibula	Fore cannon	Rear cannon	Average
		pounds	pounds	pounds	pounds	pounds	pounds	pounds
<i>No bonemeal available</i>								
<i>Jersey</i>		330 ^a	340 ^b					335
Cow No. 59	Dairy ration, unsupplemented							
<i>Range Cows</i>								
No. 1	Range on sand and muck lands					1,530	1,998	1,764
No. 2	Range on sandy lands	2,240	2,405	1,923	2,602	1,883	2,155	2,201
No. 3	Range; peanut hay in winter					2,120	2,380	2,250
<i>Two per cent of bonemeal in concentrates</i>								
<i>Guernsey</i>								
Cow No. 297	Dairy ration; bonemeal 1 mo.							
<i>Dutch Belted Cow</i>	" " ; bonemeal 29 mo.	3,535	3,645	3,710	3,240	3,470	3,440	3,455
<i>Jersey Cows</i>								
No. 81, 120, 195, 225	" " ; bonemeal 19-27 mo.	3,244	3,636**	2,848	3,299	2,228	3,302	3,037
No. 177, 188, 218	" " ; bonemeal 13-23 mo					2,700	3,788	3,244
<i>One per cent of bonemeal in concentrates</i>								
<i>Aberdeen Angus</i>								
6 cows	Grass, silage, concentrates							
	<i>Access to bonemeal, ad libitum</i>							
<i>Steers</i>								
8 grade Angus	Grass pasture, with peanut hay in winter					2,689	3,370	3,030
16 native and grade Herefords	Grass pasture, with peanut hay in winter					2,059	3,059	2,559
						2,426	3,184	2,805

*—Breaking strengths were determined by using a 6-inch span with weight applied slowly in the middle from above, except (a) a 5-inch span, and (b) a 7-inch span with Cow No. 59.

**—One femur of Cow No. 195 not available.

Under this condition of inadequate calcium intake, not only were milk yields less than expected, but mineral reserves were depleted to the point of skeletal fragility. A significant proportion of the cows had broken ribs or hips. Since the correction of the calcium deficiency by the addition of bonemeal to the rations, the cows have suffered no broken bones in the next four years, mineral reserves (as measured by bone strengths of cows slaughtered) have been restored, and milk yields have been attained commensurate with inheritance and the organic nutrient intake of the same cows.

STATISTICAL ANALYSIS

Study of the records for possible contributing factors arising from management of the cows disclosed that the dry periods prior to lactation averaged 80 days in length preceding use of bonemeal, as against 86 days while bonemeal was available. This excludes the 12 lactations after the first parturition of each cow in the former period, and one instance of difficult conception among the 22 lactations in the latter period. The average date of conception was at 105 days after parturition in the former interval, as against 169 days (*including* five cases of difficult conception of 300 days or over) in the latter period. In addition, cystic ovaries were encountered in two old cows, and four failed to conceive. The observation of Dr. C. H. Eckles in Minnesota Station Bulletin 258, was borne out in that the older cows conceived less readily.

From statistical analysis of differences in rate of decline between milk production on the low-calcium rations, and that on the supplemented rations, it was found that the standard error of difference, divided into the difference is 1.73, or that the probabilities are 9 in 10 times that the differences are not due to chance. In other words, these differences are due to inherent differences in the rate of production rather than to chance. This took into consideration the entire lactation curves from the second to thirteenth months inclusive.

The entire lactations include the period immediately after calving, during which the mineral matter stored in the skeleton in the dry period was available. After this available supply is depleted by lactation, the cows become dependent upon the limited amounts in the feed, and milk secretion is checked. When the curves for the 7th to 13th months were analyzed similarly, the factor 72.17 was found in place of 1.73. In other words, it is highly significant that these differences are inherent, rather than due to chance.

SUMMARY AND CONCLUSIONS

Typical rations used in feeding the Jersey cows at the Florida Agricultural Experiment Station during a period of years supplied an excess of protein, energy and phosphorus, but were low in content of calcium. Addition of bonemeal as two per cent of the concentrates was sufficient to render the calcium level adequate for a commercial dairy herd.

This increase in calcium level in the rations allowed 12 Jersey cows to attain an increase of four pounds of milk per day in subsequent lactations, and to be more persistent producers throughout longer lactation periods. At the same time, these cows attained a stage of mineral storage in the skeletal tissues such that the leg bones from nine of them had average breaking strengths in excess of 3,000 pounds. On the other hand, in absence of the calcium supplement, several of these same cows previously had withdrawn mineral reserves from the skeleton to such an extent that an unusual proportion of them had suffered broken hips and ribs.

The possibility of corn silage, grown on low-calcium soils, as a source of roughage for use in studies of calcium metabolism is suggested.

ACKNOWLEDGMENTS

The lactation periods prior to May 15, 1928, were accumulated under the direction of Professor John M. Scott, formerly Animal Industrialist and vice-director of the Florida Agricultural Experiment Station. Alex R. Mathers assisted in assembling and tabulating the milk records. Professor C. H. Willoughby allowed us to obtain leg bones from Aberdeen Angus cows for use in this study. Professor Charles C. Brown of the Civil Engineering Department made available such equipment as was used in bone strength determinations. Bradford Knapp, Jr., and Dr. L. W. Gaddum assisted with the statistical analyses, testing the significance of the data presented herein.

REFERENCES

1. CLARK, R. S. The correlation between changes in age and milk production of dairy cows under other than official testing conditions. *JOURNAL OF DAIRY SCIENCE* 7: 547-554. 1924.
2. ECKLES, C. H. Some phases of the phosphorus deficiency problem in the nutrition of dairy cattle. Presented before Amer. Dairy Sci. Assoc., Washington, D. C., June 26, 1929.
3. FINGERLING, G. Beitrage zur Verwertung von Kalk- und Phosphorsäureverbindung durch den tierischen Organismus. *Landw. Vers. Stat.* 75: 1-52. 1911.
4. KELLNER, D. Die Ernährung der landwirtschaftlichen Nutztiere. Paul Parey, Berlin. 10th edit. 1924. Pages 618-619.
5. MEIGS, E. B. and WOODWARD, T. F. The influence of calcium and phosphorus in the feed on the milk yield of dairy cows. *U. S. Dept. Agr. Bul.* 945: 1-28. 1921. Also *JOURNAL OF DAIRY SCIENCE* 4: 185-217. 1921.
6. NESSLER, J. II. Untersuchungen der Knochen von Knochenbruchigkeit Rindvieh. *Landw. Vers. Stat.* 16: 187-196. 1873.
7. THEILER, SIR A., GREEN, II. H. and DU TOIT, P. J. Phosphorus in the livestock industry. *Jour. Dept. Agr. (Union of S. Africa)* 8: 460-504. 1924.
8. TUFF, P. Osteomalacia and its occurrence in cattle in Norway. *Jour. Comp. Path. and Ther.* 36: 143-155. 1923. Also *World's Dairy Congr. II*: 1494-1501. 1923.
9. WELLMAN, O. Futterungsversuche über die Bedeutung des Mineralstoffwechsels, insbesondere der Erdalkalien und Phosphorsäure. *Biologia Generalis*, 8: 387-396. 1922.

THE DETECTION OF THE ESCHERICHIA-AEROBACTER GROUP IN BUTTER

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A plentiful supply of pure water is a valuable asset in the manufacture of butter of good quality; and therefore the employment by the dairy bacteriologist of a simple routine method for the detection of the Escherichia-Aerobacter group in butter is useful as a guide to the character of the water used for washing the butter during the process of manufacture.

Hammer and Yale (1) have shown that the growth of the Aerobacter species is more injurious to the keeping quality of the butter than the growth of the Escherichia species; that the Aerobacter species when butter was held at 18° C. regularly developed off odors and flavors in either salted or unsalted butter.

An old method for the detection of the Escherichia-Aerobacter group was the use of litmus lactose bile medium, but it is known that this medium favors the growth of the Escherichia group; and in "Standard methods of Water Analysis, 1925," it is recommended that a standard lactose broth medium be used. If there is formation of 10 per cent or more of gas in a standard lactose broth fermentation tube within 24 hours at 37° C., it is recommended to make one or more Endo- or Eosin-Methylene-blue plates from the tube which shows gas formation from the smallest amount of water tested, and to note the type of growth when the Petri dishes are incubated at 37° C. for 24 hours. Absence of gas formation after 48 hours' incubation constitutes a negative test, and there is a note—"an arbitrary limit of 48 hours' observation doubtless excludes from consideration occasional members of the Coli-Aerogenes group which form gas very slowly, but for the purpose of a standard test the exclusion of these occasional slow gas-forming organisms is considered immaterial."

The presence and detection of these slow gas-forming organisms is of importance to the dairy bacteriologist, since there are members of the subgenus Aerobacter whose optimum temperature is about 25° C. and which give only scanty growth when grown at 37° C. on standard lactose nutrient agar, and show little or no gas formation when grown in standard lactose broth solution and held at 37° C. for a period of 24 to 48 hours, while they produce a marked amount of gas in the fermentation tube when

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held at 21° C. to 25° C. for 24 hours. The detection of the *Aerobacter* Group in butter is of importance, since these originate from soil, grains or feeds, and if found in butter their presence shows contamination of the water used at the creamery, or of the cream after pasteurization.

Various workers have found that lactose peptone broth solution is not a reliable enrichment medium for the *Escherichia-Aerobacter* group. This has led to the use of dyes having a selective bactericidal action and we have the Gentian violet lactose peptone bile broth of Kessler and Swenarton (2), the Trypaflavin lactose broth of Klimmer and coworkers (3), and the Brilliant green bile broth of Muer and Harris (4). Solid media for direct estimation such as Eosin methylene-blue agar have been used for direct count; but for routine work, where the percentage of positive results is not high, as in the bacteriological analysis of butter, a simpler method of detecting the presence of the *Escherichia-Aerobacter* group is desirable.

In the routine examination of butter in our laboratory it is usual to add one gram of the emulsified butter to 9 cc. of warm water, and, after mixing thoroughly, to pipette 1 cc. of the dilution into each of two fermentation tubes containing standard lactose broth solution and two tubes of sterile litmus milk. One of each pair is inoculated at 21° C. and the other two at 37° C. Results obtained over a period of several years have shown that at times gas will be produced at 21° C. in both fermentation tube and litmus milk when there is no evident gas formation at 37° C., and streaking on Eosin-Methylene-blue plates shows the presence of members of the subgenus *Aerobacter*. It has been noticed that coagulation of the milk with acid and gas formation occurs at times at both 21° C. and 37° C. when there has been no evident gas formation in the fermentation tube, and when a portion of the milk serum was streaked on an Eosin-Methylene-blue plate, a typical *Escherichia* or *Aerobacter* growth was obtained.

It is recommended, therefore, in the routine examination of butter to follow the usual procedure of placing the sample bottle containing the butter to be tested, in a water bath held at 40° C. to 45° C. and that the sample be shaken until the butter is of a thin creamy consistency. In addition to carrying out the ordinary bacteriological analysis, one cc. of the melted butter is added to 9 cc. of sterile water held at 40° C. to 45° C. and the contents thoroughly mixed. One cc. of this dilution is then added to two tubes of sterile litmus milk. One tube is incubated at 21° C. and the other test-tube at 37° C.

If there is coagulation of the litmus milk with production of acid and gas, the positive tube is streaked on an Eosin-Methylene-blue plate to confirm the presence of the *Escherichia-Aerobacter* group. In our work the litmus milk has proved a better enrichment medium than nutrient lactose broth for the detection of the *Escherichia-Aerobacter* group in butter.

REFERENCES

- (1) HAMMER, B. W., AND YALE, M. W. Development of the Escherichia-Aerobacter group of bacteria in butter. JOURNAL OF DAIRY SCIENCE 15: No. 3, p. 199. 1932.
- (2) KESSLER, M. A., AND SWENARTON, J. C. Gentian violet lactose peptone bile for the detection of *B. coli* in milk. Jour. Bact. 14: No. 1, p. 47. 1927.
- (3) KLIMMER, M., HAUPT, H., AND BORCHERS, F. Über das Vorkommen und die Bestimmung der Coli und Aerogenesbakterien in der Milch. Milchwirtsch. Forschgn. 9: p. 236. 1930.
- (4) MUER, T. C., AND HARRIS, R. L. The value of brilliant green in eliminating errors due to anaerobes in the presumptive test for *B. coli*. Amer. Jour. Pub. Health 10: p. 874. 1920.

STUDIES ON THE IODINE CONTENT OF MILK

I. EFFECT OF DESICCATION AND STORAGE

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The importance of small amounts of iodine in nutrition seems to have been fully demonstrated by numerous investigators. Such data as are available indicate that milk normally contains small and highly variable amounts of iodine; the amounts reported ranging from traces to 276 parts per billion (1, 2, 3, 4 and 5). That such variations may be due primarily to the amount of iodine ingested in the ration is clearly shown by the work of Scharrer and Schwaibold (6), Krauss and Monroe (7) and by Watson (8). While the analytical difficulties involved in the quantitative estimation of the small amounts of iodine usually found in milk may preclude full acceptance of absolute values recorded, the general conclusions can hardly be contradicted.

The nature of the element and lack of knowledge regarding its exact combination in milk presents certain speculative questions as to whether iodine, either that existing as a normal constituent in milk or that which might be subsequently added, is retained after the milk is subjected to certain common procedures. Magee and Glennie (3) have reported that 20 per cent or more of the iodine content of milk is lost by heating.

Data here presented show the iodine content of milk before and after desiccation by the atmospheric double roller drying process; also the comparative iodine content of fluid and desiccated milks to which elemental iodine in suitable form and in definite amounts had been added previous to drying.

EXPERIMENTAL

The plan of experiment involved iodine determinations of parallel samples of fluid and dried milk to which elemental iodine had been added in known amounts before desiccation. It is rational to suppose that iodine added as the element would be more readily lost in the drying process than if added as iodide. This procedure was therefore used in order to make the conditions of the experiment as drastic as possible. The iodine undoubtedly unites at once to a large extent with the protein of the milk. A

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single batch of several hundred pounds of milk was divided into a number of smaller portions which were held at 70° to 75° F. for various periods of time until different degrees of acidity were developed. Portions of certain of the samples were conserved for subsequent analysis by the addition of sodium hydroxide and slow evaporation at 60° to 80° C. during a period of 24 to 36 hours, other portions of the same samples being dried by the roller process. In order to analyze milk for iodine, it is necessary that the sample be dried in some manner before combustion. Drying at 60–80°, after addition of alkali, was adopted for the control experiments as being least likely to involve losses of iodine. That iodine is not lost by heating at 80° in the presence of sodium hydroxide has been shown by Baumann (9). This parallel series of samples contained only the iodine inherent in the milk.

To other portions of milk of the same acidities, elemental iodine was added at the calculated rate of 2000 parts per billion of milk solids. This was accomplished by adding the proper amount of an assayed iodine solution directly to the milk during violent agitation and continuing the agitation for a few minutes. The strength of the iodine solution was determined by titration against N/100 sodium thiosulphate which had previously been standardized against iodine. Samples of the iodized fluid milk were conserved for later analysis in the same manner as were the uniodized samples. The remainder of the iodized samples were dried by the roller process. All desiccated samples were packed in hermetically sealed containers immediately after drying.

Although it was intended to add an exact 2000 parts of iodine per billion parts of milk solids, a careful appraisal of all possibilities of error was made for calculating the possible iodine content if all the errors were:

- (a) Acting to increase concentration of iodine in the milk.
- (b) Acting to decrease the concentration of iodine in the milk.

While it is improbable that all of the possible mechanical errors operative for a particular sample were of like action, the extremes of concentration which might exist lay between 1931 and 2174 parts per billion. The amount of iodine found in the uniodized fluid milk was taken into consideration for calculating the possible extremes of total iodine content of the samples to which iodine had been added. The calculations indicate that the total iodine content of such samples could not have been less than 2344 parts per billion nor more than 2625 parts per billion, dry basis.

A modification of the McClendon-Remington method of analysis (10, 5) was used with the exercise of such precautions as experience with the method seems to require. The Karns (11) method as modified by von Kolnitz and Remington (12) was used for a few check determinations with results substantially equivalent to those obtained by the McClendon-Remington method. The records in the accompanying table are the result of sev-

eral determinations made on each sample and include only those which showed reasonably close agreement. Low, and often entirely negative recoveries were obtained in many instances but in the majority of such cases one or more elements of mismanagement or inadvertency occurred in the manipulations which could account for the low result. It is to be noted that during the course of the work a possible serious source of error was indicated by the presence of chlorine gas in the atmosphere and by traces of chlorine in the distilled water obtained from an over-chlorinated municipal water supply. Although positive proof that such chlorine was responsible for many of the low recoveries is lacking, higher recoveries for subsequent estimations were substantially concurrent with the removal of the chlorine from the distilled water by activated granular carbon and with the elimination of chlorine from the atmosphere.

The data for comparable samples of milk dried by the commercial roller process, and the milk slowly evaporated at 60°–80° C. after the addition of alkali indicate that there is no significant loss of iodine due to the desiccating process under consideration. The percentage recovery from both series

TABLE 1
The recovery of iodine from liquid and dry milk

SAMPLE	METHOD OF DRYING	TITRABLE ACIDITY (cc. n/10 alkali per 100 cc. milk)	EXTREME LIMITS OF IODINE ADDED	EXTREME LIMITS OF TOTAL IODINE IN SAMPLE	IODINE RECOVERED	LIMITS OF RECOVERY
			<i>parts per billion, dry basis</i>			<i>per cent</i>
1	With alkali, 80°	14.8	None		392	
1A	Roller Process		None		350	89.3
1B	Roller Process	14.8	1936–2174	2347–2635	2052	78–87
2A	Roller Process	16.3	1934–2174	2344–2635	2166	82–92
3	With alkali, 80°	18.5	1931–2174	2340–2635	1952	74–85
3A	Roller Process		1931–2174	2340–2635	2074	79–89
4	With alkali, 80°	20.8	1927–2174	2336–2635	2126	81–91
4A	Roller Process		1927–2174	2336–2635	2026	77–87

of samples was substantially the same. This clearly shows that recoveries less than the calculated amount must be due to some factor or combination of factors which affect both methods of drying, such as losses of free iodine during the process of addition to the milk, or flaws in the method of analysis. Variations in titratable acidity of the fluid milk ranging from 0.148 to 0.208 per cent, calculated as N/10 acid, did not appear to affect the recovery of iodine from either of the sets of samples. Samples of the desiccated product analyzed at intervals during a storage period of seven months did not show significant differences in iodine content in comparison with results obtained soon after preparation.

TABLE 2
Effect of storage on iodine content of dry milk

TIME OF STORAGE	IODINE CONTENT
0	2052
1 month	1918-1952
4 months	2072
7 months	1900

CONCLUSIONS

1. The iodine content of milk dried by the atmospheric double roller process is not less than that of milk slowly evaporated to dryness at 60-80° C. after the addition of sodium hydroxide. An average recovery of about 83 per cent was obtained from both milks to which elemental iodine had been added prior to drying.

2. Change of acidity within a range suitable for drying milk by the roller process does not affect the recovery of added iodine.

3. No apparent loss of iodine from dry milk results from storage for as long as seven months.

REFERENCES

- (1) VON FELLEBERG, T. Untersuchungen über das Vorkommen von Jod in der Natur. VI. Mitteilung: Ueber den Zusammenhang zwischen der Häufigkeit des Auftretens von Kropf. *Biochem. Ztschr.* 152, 141. 1924.
- (2) MONROE, C. F. The possibility of producing iodized milk. *Bi-monthly Bull. Ohio Agric. Exp. Sta.* 13, No. 4: 155. 1928.
- (3) MAGEE, H. E., and GLENNIE, A. E. Studies on the effect of heat on milk. IV. The iodine content. *Biochem. Jour.* 22: 11. 1928.
- (4) ORR, J. B. and LEITSCH, I. Iodine in nutrition. *Brit. Med. Res. Council, Sp. Report*, No. 123. 1929.
- (5) McCLENDON, J. F., REMINGTON, R. E., VON KOLNITZ, H. and RUFFE, R. The determination of traces of iodine. III. Iodine in milk, butter oil and urine. *Jour. Am. Chem. Soc.* 52: 541. 1930.
- (6) SCHARER, K. and SCHWAIBOLD, J. The iodine content of milk. *Biochem. Ztschr.* 207, 333. 1929.
- (7) KRAUSS, W. E. and MONROE, C. F. A comparison of the influence of iodized milk and potassium iodide administered directly, on the size and iodine content of the thyroid gland of rats. *Jour. Biol. Chem.* 89: 581. 1930.
- (8) WATSON, C. J. A study of the reactions involved in the determination of minute traces of iodine, and the application of some feeding experiments with fishmeal. Thesis Cornell Univ. Library. 1928.
- (9) BAUMANN, E. J. and METZGER, N. The determination of iodine in blood, foods and urine. V. *Jour. Biol. Chem.* 98: 413. 1932.
- (10) REMINGTON, R. E., McCLENDON, J. F. and VON KOLNITZ, H. The determination of traces of iodine. V. Further refinements in technique. *Jour. Am. Chem. Soc.* 53: 1245. 1931.
- (11) KARNS, G. M. Ashing apparatus for samples containing traces of iodine. *Ind. and Eng. Chem., Anal. Ed.*, 4: 299. 1932.
- (12) VON KOLNITZ, H. and REMINGTON, R. E. A simplified Karns technique for the micro-estimation of iodine. *Ind. and Eng. Chem., Anal. Ed.*, 5: 38. 1933.

STUDIES ON THE IODINE CONTENT OF MILK

II. VARIATIONS IN THE MIXED MILK OF HERDS

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INTRODUCTION

When compounds of iodine are ingested by lactating animals, a part of the iodine appears in the milk. For pharmacological doses, this has been known for many years (1). That it is likewise true for those smaller amounts which occur in normal nutrition, and that the iodine of the milk is increased by an increase in that ingested, has been shown by Scharrer (2) and by Krauss (3). It seems evident that iodine is a normal constituent of milk, being correlated in amount to the available iodine content of the ration of the cows.

It has been shown by Hayne (4) that diseases due to iodine deficiency are not endemic in South Carolina, and by Remington (5) that crops which are produced there contain more iodine than those from more goitrous areas. In estimating the iodine available to the population in this area, it is necessary to include some studies on milk.

Many of the values found in the literature for the less common elements in foods (and for vitamins as well), are based on a single sample, taken without regard to locality, season, or conditions of growth. The work of the senior author (6) and his associates has brought out that wide variations exist, not alone with regard to iodine, but also with regard to iron, copper, and manganese, and not only in agricultural crops, but sea food as well (7); hence the acceptance of values based on one, or but few samples, may give rise to erroneous conclusions.

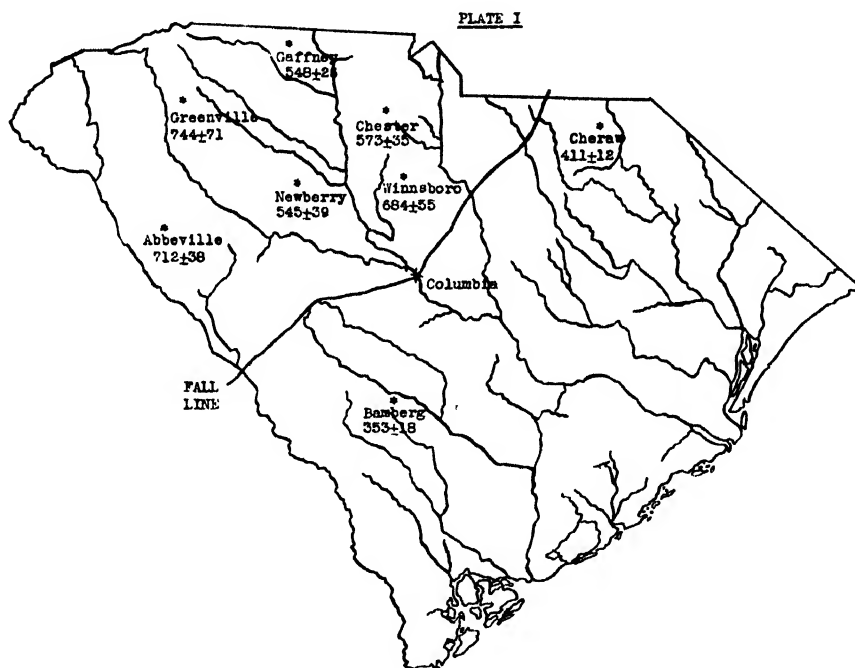
In the present investigation, the aim is to determine what variations may exist in the iodine content of the mixed milk of herds from a given area, and to correlate such variations as may be found with locality, season of the year, or any other factors which may be observed.

PLAN OF THE INVESTIGATION

Through the cooperation of the Dry Milk Company, an experimental milk drying plant was installed in the territory. This plant was of the roller process type, and operated by an experienced technician furnished

by the Company. The method of handling and drying was made to conform to that in usual commercial practise. A portion of the fat of the milk was removed prior to drying. This could not materially affect the iodine content, it having been shown by Scharrer (2) that not over 4 per cent of the total iodine of milk is normally contained in the fat. That the iodine content of milk is not materially reduced by this method of drying has been shown in the preceding paper (8).

Eight collecting points were selected, two lying near the foot of the Blue Ridge of the Appalachian Mountains, four in the rolling country between the Blue Ridge and the Coastal Plain, and two in the Coastal Plain (See map, Plate I). Milk was collected in batches of approximately 1,000



pounds, three times a week, the collecting points being handled in rotation. Each point, therefore, was visited at intervals of three weeks, one collecting date on each circuit being left vacant for shop and office work in the plant. In selecting herds for the experiment, it was specified that only cattle receiving none but home-grown feeds were desired. The infrequent collections, from any given point, and the restriction as to the use of feeds imported from other regions made it necessary to draw the samples from small herds. On this account, the number of dairymen contributing to each sample of milk ranged from seven to nineteen, as follows:

Number of herds contributing

Abbeville	19
Bamberg	7
Cheraw	14
Chester	7
Gaffney	15
Greenville	7
Newberry	7
Winnsboro	13

It was originally planned that the collection and drying of the milk samples should extend over a period of one year, but due to reasons not pertinent to this discussion, it was terminated at the end of ten months. The first collection was made on October 26, 1931, the last on September 2, 1932. Four times during the course of the experiment, a dairy specialist from the laboratory made the complete circuit, visiting every farmer, and obtained data as to methods of feeding, and samples of all feed and forage used. During the course of the project, fifteen samples were collected from each of five points, fourteen from each of the other three.

Samples of all batches of milk powder produced were sent to the laboratory as promptly as possible. Without further drying or manipulation, these were analyzed for iodine. The analytical procedure at the beginning of the study was that devised by McClendon as modified by Remington and associates (9). This method, while the best available for large samples at that time, is extremely laborious, and gives frequent failures which cannot with certainty be attributed to any known cause. In April there became available to us a manuscript by Karns (10), describing a method of ashing in a closed bulb which seemed to offer distinct advantages. There was accordingly devised a simplified type (11) of the Karns apparatus, and after experimental work on the recovery of known amounts of iodine, the method was adopted as routine on subsequent samples. Following this change, all analyses previously made by the McClendon method were repeated, using the newer technique, with the result that the average recovery of iodine was about ten per cent higher than had previously been obtained.

Since the method of final estimation is an isolation and colorimetric one, and since possibility of contamination by reagents has been rigorously ruled out, the only errors possible are those due to loss of iodine in the process of burning and subsequent manipulation of the samples. The *highest value* that can be reproduced within a maximum limit of variation of ten per cent on a repeat analysis, is, therefore, taken as the most nearly correct.

GENERAL RESULTS

The analytical work embraced 117 samples from the eight collecting points in South Carolina, 9 samples during the same period taken from the regular run of a commercial dry milk plant at Bainbridge, N. Y., and 6

TABLE 1

Iodine content of milk samples, collected in South Carolina, arranged as to points and dates of collection

(Parts per Billion, Dry Basis)

	ABBEVILLE	RAYBERG	CHERAW	CHESTER	GAFFNEY	GREENVILLE	NEWBERY	WINNSBORO	MEAN	P.E.M.
Oct. 26-Nov. 13	828	336	370	892	804		338	724	613	± 64
Nov. 16-Dec. 4	616	384	456	436	680	628	447	885	566	± 59
Dec. 7-Dec. 26	828	456	476	672		600	472	844	621	± 42
Dec. 28-Jan. 15	852	412	349	546	668	424	576	837	583	± 45
Jan. 18-Feb. 5	424	412	444	584	376	466	468	1041	527	± 52
Feb. 8-Feb. 26	668	428	363	536	652	457	592	917	577	± 38
Feb. 29-Mar. 18	624	440	464	758	556	585	1080	1432	742	± 75
Mar. 21-Apr. 8	588	264	432	1080	600	492	1056	668	648	± 69
Apr. 11-Apr. 29	552	440	432	400	472	492	500	496	473	± 11
May 2-May 20	474	224	300	333	500	496	446	497	409	± 26
May 23-June 10	548	143	376	428	420	840	428	324	438	± 47
June 13-July 1	1120	244	324	684	279	1084	311	356	550	± 87
July 2-July 22	636	440	520	420	536	972	484	492	563	± 42
July 25-Aug. 12	760		492	432	452	1008	420	388	565	± 59
Aug. 15-Sept. 2	1170	320	360	400	680	1872	560	360	715	± 130
Mean	712	353	411	573	548	744	545	684	572	
P.E.M.	± 38	± 18	± 12	± 35	± 26	± 71	± 39	± 55		± 16

TABLE 2

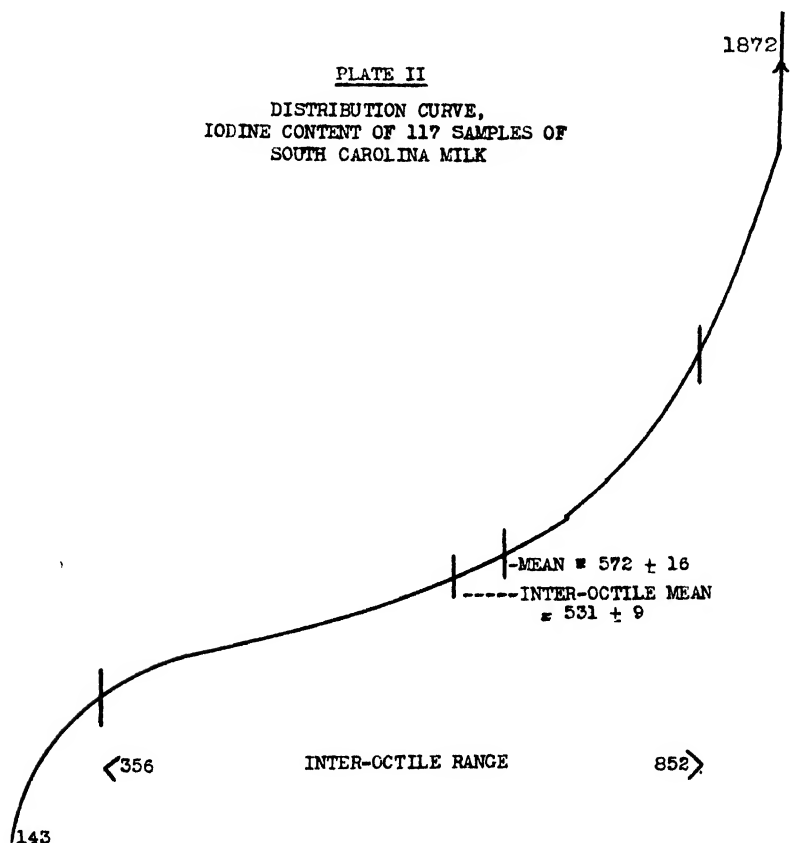
Iodine content of milk produced at Bainbridge, N. Y., and Columbus, Wisconsin
(Parts per Billion, Dry Basis)

BAINBRIDGE		COLUMBUS	
date	iodine	date	iodine
1-16-32	389	1- 7-30	388
4- 8-32	235		
4-15-32	245	4- 8-30	316
4-22-32	335	4-17-30	395
6-10-32	131	6-16-30	171
9-10-31	157	9- 9-30	338
9-11-31	147		
12-21-31	392	12- 7-30	326
12-17-31	350		

samples similarly obtained at Columbus, Wisconsin. The South Carolina samples, as shown in detail in table 1, ranged in iodine content from 143 to 1,872 parts per billion, with a mean value of 572 ± 16 .

The distribution curve is shown in Plate II. If the upper and lower octiles be discarded, the range is from 356 to 852, mean 531 ± 9 .

The range of values from Bainbridge, N. Y. (Table 2), was 392 to 131, mean 265, and from Wisconsin 395 to 171, mean 322.



RELATION TO PLACE OF PRODUCTION

The mean of all samples from each collecting point has been determined, and treated statistically according to accepted methods (see Sherman, Chemistry of Foods and Nutrition, Appendix D, for a simple explanation) in order to determine the probable errors of the means, and significance ratios for their differences. In table 3, the upper figure in each block is the difference between the average values for the two points concerned, the lower figure the significance ratio obtained as above, from which it becomes apparent that while Cheraw and Bamberg are scarcely significantly

different from each other, they are both significantly lower than all other points.

TABLE 3

Significant data for average iodine content of milk from eight collecting points

The first figure in each square is the difference between the average values for the two points shown; the second, the significance ratio obtained by dividing this difference by the square root of the sum of the squares of the probable errors of the two averages.

	BAMBERG	CHERAW	CHESTER	GAFFNEY	GREENVILLE	NEWBERRY	WINNSBORO
ABBEVILLE 712 \pm 38	359 8.5	301 7.5	139 2.7	164 3.6	32 0.4	167 3.1	28 0.4
BAMBERG 353 \pm 18		58 2.6	220 5.6	195 6.1	391 5.3	192 4.5	331 5.7
CHERAW 411 \pm 12			162 4.4	137 4.7	333 4.6	134 3.3	273 4.9
CHESTER 573 \pm 35				25 0.6	171 2.2	28 0.5	111 1.7
GAFFNEY 548 \pm 26					196 2.6	3 0.1	136 2.2
GREENVILLE 744 \pm 71						199 2.5	60 0.6
NEWBERRY 545 \pm 39							139 2.1
WINNSBORO 684 \pm 55							

The two points showing highest values, Abbeville and Greenville, are scarcely significantly different from other points except Bamberg and Cheraw, with the possible exception of the difference between Abbeville and Gaffney, which shows a ratio of 3.6.

This higher iodine content of milk from points in the Piedmont as compared with the Coastal Plain, is in agreement with previously published data on potatoes (5), and with many unpublished results on leafy vegetables. It apparently contradicts the salt spray theory of iodine distribution, and suggests a source of iodine enrichment inherent in the origin of soils in this region.

RELATION TO SEASON

Inspection of average values for individual collection periods (Table 1) reveals a rather definite fall in iodine content during April and May, fol-

lowed by recovery in mid-summer. This period of lower values covered three circuits, from April 11 to June 10. If these three circuits are averaged together, the preceding eight circuits averaged in two groups of four each, and the following four circuits treated as one group, the following mean values are obtained:

Oct. 26-Jan. 15	30 samples	595 \pm 23
Jan. 18-Apr. 8	32 samples	623 \pm 32
Apr. 11-June 10	24 samples	440 \pm 33
June 13-Sept. 2	31 samples	599 \pm 43

There appears to be a significant difference between the second and third groups (ratio 3.9) and between the third and fourth (ratio 2.9). If, however, we discard the highest and lowest octiles and then treat the data in the same way, the following results are obtained:

Oct. 26-Jan. 15	25 samples	601 \pm 22
Jan. 18-Apr. 8	25 samples	523 \pm 14
Apr. 11-June 10	19 samples	486 \pm 15
June 13-Sept. 2	20 samples	494 \pm 18

The only significant difference here is that between the first group and each of the others. Probably about all we can say is that there is a slight definite decrease in iodine content in the spring, followed by a return to higher values in late summer or fall.

Of the 9 samples from Bainbridge, the highest values were obtained in December and January, the lowest in mid-summer (June to September), and the difference is much greater than in the South Carolina series. If a similar drop occurred at Columbus, it is evidenced by only one sample (June) of the six analyzed. Although but few analyses are given for these northern points, they represent large batches from many producers, and hence should be compared with averages rather than individual values in the South Carolina series.

The results indicate that the iodine content of milk in South Carolina is higher throughout the year and less subject to seasonal fluctuation, than that of Central New York or Wisconsin.

RELATION TO DIET

In an attempt to find a relationship between the iodine content of the milk and that of the ration, each South Carolina producer was visited four times during the collection period, *viz.*, in January, March, May, and August. At each visit, samples were taken of all materials fed, and information obtained as to kind of salt used, and source of water supply. From the samples obtained, and making use of information gained at time of collection, a composite sample representing the complete ration of each herd was constructed. These were later combined, in proportion to the amount

of milk contributed by each herd to a single batch of milk, so as to make a feed sample which corresponded to the milk sample for the given place and date. There were thus obtained 32 composite feed samples, 4 from each point, which could be compared as to iodine content with the milk samples.

Of the 89 herds, 3 received rock salt, 12 Morton's Stock Salt (non-iodized), and the balance common salt. The water supply was almost universally from surface waters, 75 per cent being from springs, streams and ponds, and 25 per cent from shallow wells. In January six herds in five localities received commercial mixed feeds in small amount. In two of these the formula showed 1 per cent of iodized salt. The number using commercial feeds fell to five in May, and three in March and August.

The principal concentrate used generally was cottonseed or cottonseed products, which for all herds amounted to 24 per cent of the dry weight of the ration in January, 27 per cent in March, 18 per cent in May, and 15 per cent in August. Next in importance among the concentrates was corn, accounting for 9, 8, 4, and 3 per cent of the ration respectively in the different periods. Legume hays, mainly pea and soy bean, were fed to the extent of 29, 23, 4, and 3 per cent, and other roughages, mostly corn and sorghum hay and silage 34, 34, 15, and 10 per cent. Although cows range out of doors the year round, no grazing was reported in January, 5 per cent in March, 58 per cent in May, and 63 per cent in August.

During the period when fresh pasturage contributed materially to the ration, all but a very few herds were on permanent pasture, the basis of which was Bermuda grass, usually with one or more legumes. In May 65 per cent of the pastures contained, in addition to grasses, some form of clover, such as burr, hop, or white clover, lespedeza or alfalfa, many containing several varieties together. In August, on the other hand, 82 per cent of pastures contained legumes, in this case mostly lespedeza.

The average iodine content of the 32 rations was found to be 475 parts per billion, but the range of values is almost as wide as for the milk samples. While those feed samples showing very high values produced milk of similar iodine content, in general no correlation could be found between the feed and the milk. In fact, it is apparent that the experiment is not sufficiently controlled, since we have been forced to rely on the statements of herdsmen as to the qualitative and quantitative make-up of the ration; the factor of accidental contamination of ration or milk (as, for instance, by the use of tincture of iodine as an antiseptic on the person, or on cows themselves) has not been guarded against; and no account could be taken of physiologic factors which might influence the metabolism of iodine by the animals.

A number of random samples of feeds, taken from different parts of the area studied, however, reveal that the legume hays are richest in iodine, and that grain in general is very poor (Table 4).

TABLE 4
Iodine content of some feeds produced in South Carolina

FEED	NO. SAMPLES	IODINE PARTS PER BILLION DRY BASIS
Concentrates		
Cottonseed Meal	4	151
Corn	3	10
Oats	1	20
Wheat	2	17
Legume Hays		
Peavine	11	522
Soy Bean	14	526
Lespedeza	2	446
Alfalfa	7	429
Vetch	8	425
Sweet Clover	2	320
Other Hays and Roughage		
Cottonseed Hulls	5	107
Oat Hay	2	116
Mixed Meadow Hay	3	263
Cane Stover	1	340
Corn Stover	1	161

Although the number of samples reported in this table is insufficient for firm conclusions, it appears probable that legume pasture and hay is the largest contributor of iodine to the ration of dairy cows in this locality.

SUMMARY

Samples of mixed milk of several herds at each of eight different points in South Carolina, were collected at intervals of three weeks, over a period of ten months (November, 1931, through August, 1932) and dried by the roller process. The cattle received only locally grown foods and feeds, and none were given iodized salt.

Iodine estimations on the dried milk were made by the method of McClendon as modified by Remington, and also by von Kolnitz' adaptation of the Karns technique, the latter method proving shorter and more workable, besides giving about 10 per cent higher recoveries.

The average iodine content of the 117 samples was 572 ± 16 parts per billion, dry basis. Averages for two points in the coastal plain (Bamberg 353 ± 18 , Cheraw 411 ± 12) were significantly lower than for six points in the Piedmont (Abbeville 712 ± 38 , Chester 573 ± 35 , Gaffney 548 ± 26 , Greenville 744 ± 71 , Newberry 545 ± 39 , and Winnsboro 684 ± 55). Values obtained in April and May were slightly but significantly lower than for the remainder of the period.

During the same period nine samples taken at a commercial milk drying plant in New York, and six samples at one in Wisconsin, averaged respectively 265 ± 24 , and 322 ± 22 ; and seasonal variations were much greater than for the South Carolina samples.

REFERENCES

- (1) KOLDA, J. Du passage des substances medicamenteuse dans Le Lait. *Le Lait* **6**: 12 and 269. 1926.
- (2) SCHARREER, K., and SCHWAIBOLD, J. The iodine content of milk. *Biochem. Zeit.* **207**: 333. 1929.
- (3) KRAUSS, W. E. A comparison of the influence of iodized milk and potassium iodide administered directly, on the size and iodine content of the thyroid gland of rats. *J. Biol. Chem.* **89**: 581. 1930.
- (4) HAYNE, J. A. Endemic goiter and its relation to the iodine content of food. *Amer. J. Public Health* **19**: 1111. 1929.
- (5) REMINGTON, R. E., CULP, F. B., and VON KOLNITZ, H. The potato as an index of iodine distribution. *J. Amer. Chem. Soc.* **51**: 2942. 1929.
- (6) REMINGTON, R. E., and SHIVER, H. E. Iron, copper and manganese content of some common vegetable foods. *J. Assoc. Official Agric. Chemists* **13**: 129. 1930.
- (7) COULSON, E. J., LEVINE, H., and REMINGTON, R. E. Oysters and Anemia. *Amer. J. Public Health* **22**: 1141. 1932. (Also unpublished data on the iodine content of oysters.)
- (8) HANFORD, Z. M., SUPPLEE, G. C., and REMINGTON, R. E. Studies on the iodine content of milk. I. Effect of desiccation and storage. *JOURNAL OF DAIRY SCIENCE* **17**: 15-18. 1934.
- (9) REMINGTON, R. E., McCLENDON, J. F., and VON KOLNITZ, H. The determination of traces of iodine. V. Further refinements in technique. *J. Amer. Chem. Soc.* **53**: 1245. 1931.
- (10) KARNS, G. M. Ashing apparatus for samples containing traces of iodine. *Ind. and Eng. Chem., Anal. Ed.*, **4**: 299. 1932.
- (11) VON KOLNITZ, H., and REMINGTON, R. E. A simplified Karns technique for the micro-estimation of iodine. *Ind. and Eng. Chem., Anal. Ed.*, **5**: 38. 1933.

SUBSTANCES ADSORBED ON THE FAT GLOBULES IN CREAM AND THEIR RELATION TO CHURNING. III. ANALYSIS OF THE ADSORBED PROTEIN*

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Protein constitutes the major part of the material adsorbed on the surface of the fat globules in cow's milk (1). The protein has been identified by various workers as a glycoprotein (2), casein (3), albumin (4), a globulin-like protein (5), and a new milk protein (6).

Some of the observed difference in composition may have resulted from the method used in the isolation of the material. A method whereby the stabilized fat globules in milk are freed from milk plasma by allowing the globules to rise through a single comparatively short column of water does not prevent the occlusion of plasma protein by the clusters of fat. A much more drastic procedure was used in the preparation of the material analyzed in the present study. The detailed method has been described in two preceding papers (1, 7). The general procedure was as follows.

Fresh heavy cream was repeatedly diluted with distilled water and passed through a centrifugal separator until the "skim milk" or wash water was free from milk plasma solids. The so-called washed cream was then churned to remove the major part of the fat. The butter was melted and the material which had remained adsorbed on the surface of the fat during the washing operation was recovered in the buttermilk and aqueous layer from the melted butter. These were concentrated and the protein fraction precipitated from the concentrate at pH 3.9-4.0. After filtration the protein was exhaustively extracted with alcohol, chloroform, and ether.

The results of analysis for total nitrogen, sulfur, and phosphorus have been reported (7). The following table also shows the nitrogen distribution analysis. The standard Van Slyke procedure described by Morrow (12) was followed. Corrections for the solubility of the basic phosphotungstates were not made inasmuch as they were filtered at a temperature of 40° F. For comparative purposes, the analyses of other milk proteins are included in the table.

It will be noted from the table that the "membrane" protein from washed cream differs from other milk proteins in all respects. The most striking difference lies in the nitrogen fractions. In this regard, it agrees

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Composition of Milk Proteins

NITROGEN DISTRIBUTION	MEMBRANE PROTEIN			CASEINA	LACT-ALBUMINA	GLOBULINA	ALCOHOL-SOLUBLE PROTEIN		HAPTEIN (6)
	I	II	Ave				(9)	(10)	
Ammonia-N	6.35	6.73	6.54	10.36	8.25	7.64	1.57	10.9	9.98
Insoluble humin-N	3.22	3.23	3.23	1.90	2.32	2.16	.24	.35	3.48
Soluble humin-N	0.43	0.44	0.44						
P.T.A. humin-N		0.20	0.20						
Total humin-N	3.85	3.87	3.86						
Arginine-N	15.54	16.09	15.82	7.90	7.38	10.81	5.98 ^b	8.30	12.79
Cystine-N	0.78	0.65	0.72	0.81	1.74	1.90			5.34
Histidine-N	2.26	1.11	1.69	5.85	4.51	3.99	3.93 ^b	2.00	5.44
Lysine-N	11.01	11.97	11.49	9.26	12.39	8.66	4.87 ^b		6.11
Basic NH ₂ -N	16.43	17.02	16.73						
Basic non-NH ₂ -N	13.16	12.80	12.98						
Total basic-N	29.59	29.82	29.71	23.82	26.02	25.35			29.68
Non basic NH ₂ -N	56.41	56.58	56.50	56.74	60.92	62.82			55.13
Non basic non-NH ₂ -N	3.55	3.46	3.51	7.50	2.33	1.14			1.61
Total non basic-N	59.96	60.04	60.01	64.22	63.25	63.96			56.74
Total recovery	99.75	100.46	100.12	100.32	99.83	99.10			99.88
Percentage total N	11.84	12.64	12.22	15.67	15.43(11)	15.44(11)	15.71	15.49	12.05
Percentage total S	0.90	1.02	0.96	0.72	1.92	0.86	.95		2.58
Percentage total P	0.24	0.51	0.33	0.85	trace	0.24	.08	.108	

^a Average of summarized data (8).^b Calculated from percentage of amino acid reported present.

with haptin, the "membrane" protein isolated by Hattori (6). Haptin and the washed cream protein are low in the percentage of total nitrogen and high in the percentage of humin or melanin nitrogen and total basic nitrogen as compared with casein, lactalbumin and globulin. The former two proteins, however, differ greatly in the nitrogen distribution within the basic fraction. Haptin is characterized by a high percentage of arginine and cystine whereas the washed cream protein is characterized by a high percentage of arginine but low percentages of cystine and histidine. The total sulfur content of haptin is also high compared with other milk proteins.

It appears further from the physical properties of these two proteins (1, 6) that they are very nearly alike but differ somewhat in composition due to impurities which have resulted from two widely different methods of preparation. Moreover, the analyses show that the "membrane" protein of milk fat is not identical with casein, lactalbumin or globulin, or the alcohol soluble protein of milk.

The serological properties of the "membrane" protein are being investigated by Dr. Julian H. Lewis, Department of Pathology, University of Chicago. A preliminary report of this study was given at the Cincinnati meeting of the American Society of Experimental Pathology, April 10-12, 1933, by L. S. Palmer and Julian H. Lewis, under the title "The Serological Properties of a New Protein from Milk." The experiments reported indicate that the protein possesses specific biological properties.

REFERENCES

- (1) PALMER, L. S. and WIESE, H. F. Substances adsorbed on the fat globules in cream and their relation to churning. II. The isolation and identification of adsorbed substances. *JOURNAL OF DAIRY SCIENCE* 16: 41. 1933.
- (2) STORCH, V. Consistensfejrt hos Smørret. Den kgl. Veterinære Landhøjskoles Laboratorium for landøkonomiske Forsøg. 36 Beretning. Ueber den Bau der Milchkügelchen. Abschnitt III Jahresber. Fortschritte Tierchem. 27: 273. 1897.
On structure of "fat globules" in cow's milk. *Analyst* 22: 197.
- (3) TITUS, R. W., SOMMER, H. H. and HART, E. B. The Nature of the protein surrounding the fat globules in milk. *Jour. Biol. Chem.* 76: 237. 1928.
- (4) BÉCHAMP, A. Sur les altérations spontanées du Lait et sur celles que la cuisson lui fait subir. *Bul. Soc. Chim.* 3rd Ser. 15: 426. 1896.
- (5) PALMER, L. S. and SAMUELSSON, E. The nature of the substances adsorbed on the surface of the fat globules in cow's milk. *Proc. Soc. Exp. Biol. Med.* 21: 537. 1924.
- (6) HATTORI, K. Über die Hüllensubstanz der MilCHFettkügelchen. *Jour. Pharm. Soc. Japan* No. 516, 123. 1925.
Beiträge zur Untersuchung über die Hüllensubstanz der MilchFettkügelchen. *Jour. Pharm. Soc. Japan* 49: 332. 1929.
HATTORI, K. and OGIMURA, Y. Beiträge zur Untersuchung über die Hüllensubstanz der MilCHFettkügelchen. II. *Jour. Pharm. Soc. Japan* 49: 1147. 1929.

- (7) WIESE, H. F. and PALMER, L. S. Substances adsorbed on the fat globulins in cream and their relation to churning. I. The churnability of artificial emulsions prepared with known emulsifying agents of cream. *JOURNAL OF DAIRY SCIENCE* 15: 371. 1932.
- (8) LAMOUR, R. K. Correlations between the total nitrogen of the bases and arginine nitrogen and between total nitrogen of the bases and lysine nitrogen, of various proteins. *Trans. Roy. Soc. Can.* 22: 349 (section 5). 1928.
- (9) OSBORNE, T. B. and WAKEMAN, A. J. Some new constituents of milk. A new protein, soluble in alcohol. *Jour. Biol. Chem.* 33: 243. 1918.
- (10) LINDERSTRØM-LANG, K. Studies on casein. III. The fractionation of casein. *Comp. rend. trav. lab. Carlsberg* 17 No. 9. 1929.
- (11) OSBORNE, T. B. and WAKEMAN, A. J. The proteins of cow's milk. *Jour. Biol. Chem.* 33: 7. 1918.
- (12) MORROW, C. A. Biochemical laboratory methods for students of the biological sciences. John Wiley and Sons, Inc., New York. 1927.

A STUDY OF THE RELATIONSHIPS BETWEEN HYDROGEN ION CONCENTRATION, TITRATABLE ACIDITY, AND QUALITY IN CHEDDAR CHEESE

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The manufacture of sour or acid cheese causes serious losses each year to the American cheese industry. The excessive acidity may affect any or all of the properties of the cheese, such as flavor, body, texture, color and finish. The defect may be almost negligible or it may be highly injurious to the market value of the product.

Excessive acidity may be avoided by skillful operators through careful observation of the milk and curd from day to day. Tests which indicate, but do not measure acidity accurately, are the so-called "rennet test" and "hot-iron test." These methods of detecting acidity attain their greatest value when used every day by the same operator on the same milk supply. The alkali titration test is commonly used to measure acidity during the cheese-making process. Optimum degrees of titratable acidity in the starter, milk or whey have been indicated for nearly every operation. But defective cheese is sometimes made even though these schedules of optimum titratable acidities have been carefully observed.

It is well known that the titratable acidity of fresh, normal milk may vary through wide limits (1) due to the influence of a number of factors (2, 3, 4). The differences in titratable acidity which may be caused by the variations in the composition of milk should be particularly significant in the manufacture of cheese. Measurements of titratable acidity during the making process actually indicate, that the maximum acidities, which may be safely attained in the manufacture of cheese from milk with high solids content, may be disastrous if applied to milk which is low in solids-not-fat.

The hydrogen ion concentration furnishes an indication of changes in milk which can not be definitely measured by titratable acidity. For example, the maximum hydrogen ion concentration attained during the fermentation of milk by a given organism is quite constant; then, also, the changes in the casein of milk, which are caused by increasing acidity, culminate in coagulation at the iso-electric point of pH 4.7. It seems possible that measurements of hydrogen ion concentration might furnish a better means of detecting critical changes during the making of cheese than do measurements of titratable acidity.

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It is the purpose of this paper to report the results of a study of the titratable acidity and hydrogen ion concentration during the making of cheddar cheese and to indicate their relation to the quality.

Determination of hydrogen ion concentration

The hydrogen ion concentration measurements of the milk and whey were made with the Biilmann type of quinhydrone electrode. This electrode was adopted because only a small sample is required and the reading may be taken immediately. These qualifications are highly desirable in as much as the amount of material available for testing is limited at times during the process and because the acidity changes so rapidly that any delay in obtaining the measurement would introduce errors. A Leeds-Northrup, type K, potentiometer and a 0.1 normal calomel electrode were used in this study. All readings were made at 25° C.

The quinhydrone electrode described and used by Knudsen (5) and Watson (6) was adopted for use in this study for determining the pH of the cheese. Some difficulty was experienced with this electrode when it was first tried.¹ There was considerable "drifting" of the potential at times, especially with fresh curd, which made it impossible to take the reading with any degree of certainty. Special precautions were taken (7) to clean the electrode properly, but even this treatment did not eliminate the trouble entirely. Finally, it was found that the addition of a drop or two of distilled water to the mixture of about two grams of cheese and 50 milligrams of quinhydrone crystals eliminated the trouble with drifting potentials.

The hydrogen electrode was also used in attempts to measure the pH of the cheese. Samples of the cheese were ground and diluted with distilled water in the proportions of 1 part of cheese to 2, 5, 10 and 20 parts of water. The results were not entirely satisfactory because the degree of dilution had a marked effect on the pH value. The measurements obtained by diluting samples of several lots of cheese of different ages are shown in figure 1. The points on these curves which show no dilution were determined by the quinhydrone method. All other measurements were made with the hydrogen electrode.

Figure 1 indicates that the degree of dilution has a distinct influence on the pH value;—the greater the dilution, the higher is the pH. The change in pH caused by dilution is variable and apparently cannot be predicted from a single observation of an unknown sample of cheese. The measurements of the samples with the least dilution (1: 2) were made with diffi-

¹ The authors are grateful for the valuable assistance and advice of Dr. H. H. Sommer, of this University, Dr. Paul F. Sharp, of Cornell University, and Dr. P. D. Watson, of the Bureau of Dairy Industry, U. S. Department of Agriculture, in correcting this difficulty.

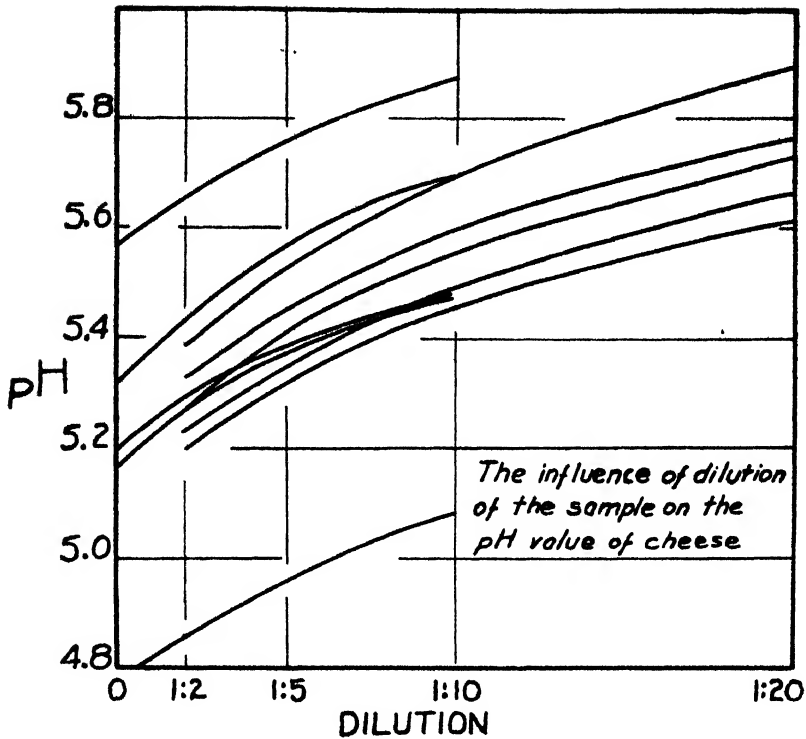


Figure 1

culty. The use of the hydrogen electrode seemed to be entirely unsuited for the purpose of this study.

Schulz (8) and Pasztor (9) have observed the same influence of dilution upon the pH of cheese. Schulz found no change in pH with the quinhydrone electrode as long as the dilution was kept below the ratio of 1:1. Pasztor reported an increase of approximately 0.50 pH between a dilution of 1:1 and 1:24.

Samples which were taken from various parts of the same cheese showed variations of not more than 0.02 pH when tested by the quinhydrone method. Some typical results of such trials are shown in table 1. An ordinary cheese trier was used to remove the sample and the half inch which included the rind was replaced in the cheese. The rest of the plug was ground in a mortar and a portion of the mixture used for the test. Schulz (8) observed a wide difference between the reaction of different parts of the interior of Tilsit cheese. These variations can probably be explained by the practice of salting this cheese from the outside and curing it under conditions which are not typical of cheddar cheese.

Determination of titratable acidity in milk, whey and cheese

The determination of titratable acidity in the milk and whey were made by the usual method of titrating 9 or 18 gram samples with 0.1 normal sodium hydroxide using phenolphthalein as indicator. The acidity was expressed as per cent lactic acid.

The A. O. A. C. method was used for determining the acidity of the cheese (10).

TABLE 1
Variations in the pH of samples taken from different portions of the same cheese

CHEESE NO.	AGE	LOCATION OF SAMPLE IN CHEESE			
		Center	Three points near the edge		
			1	2	3
	<i>days</i>	<i>pH</i>	<i>pH</i>	<i>pH</i>	<i>pH</i>
51	2	4.99	4.99	4.98	4.97
83	6	4.90	4.91	4.91	4.89
28	21	5.08	5.08	5.08	5.08

Manufacture of the experimental cheese

Paired lots of cheese were made from identical milk. One lot was made in a normal way while the second lot was manufactured in some manner to produce a sour cheese. A total of 54 lots were made in this manner. They were cured at a temperature of about 45° F.

The hydrogen ion concentration and titratable acidity measurements were made at the critical points of the curd-making process. These points included the times of receiving the milk, adding the rennet, cutting the curd, beginning and ending of the heating process, removing the whey, and packing, milling and pressing the curd. The pH and titratable acidity measurements were made on the milk until it was coagulated. When it was necessary to do so, the soft coagulum was used for pH measurements until the curd was cut. All acidity measurements were made on the whey between the operations of cutting and milling the curd. Determinations of pH were made on the curd at milling and pressing.

Acidity and pH measurements were made on the cheese when it was removed from the press, once each day for 7 days after pressing, and then at the ages of 14, 21, 35, 49 and 70 days, and approximately 9, 12 and 24 months.

The cheese were scored after 2 months of curing. The score card allowed 30 points for flavor, 40 for body and texture, 10 for color, and 20 for make-up. The scores of three judges were averaged to obtain the recorded score for each cheese. The cheese were scored again in the same manner at approximately 10 months.

EXPERIMENTAL RESULTS

The 54 lots of cheese were divided into three groups according to their quality at two months of age. Group I consisted of 14 lots scoring 90 or above; group II contained 25 lots scoring from 87 to 90, while group III was composed of 15 lots with scores of less than 87. At two months of age the average scores of the cheese in groups I, II and III were 91.1, 88.7, and 84.8, respectively. After 10 months of curing the average scores of the cheese in these groups named in the same order were 90.2, 88.7, and 86.3. These figures indicate some deterioration in the average quality of the cheese in group I and improvement in quality of the cheese in group III.

It is believed from a careful study of the ripening of cheddar cheese that the scores at the age of two months represent more accurately than the 10 months' score the influence of acidity on the properties of the cheese. After 10 months of curing, the changes in the cheese which are caused by those organisms and enzymes that are responsible for protein decomposition, tend to disguise the effects of excess acid.

Acidity measurements during the curd-making process

The average pH values at the various steps in the curd-making process are shown with their respective probable errors in table 2. These average values were calculated directly from the pH measurements. Averaging the pH values gave practically the same result as would have been obtained if the acidity had been stated in terms of hydrogen ion concentration and these values then averaged and the average converted into pH. For example, the pH measurements of 13 lots of cheese at the intervals of cutting, 3 days after pressing and 9 months after making, were averaged separately and found to be 6.473, 5.045 and 5.323, respectively. The same acidity measurements were then converted to hydrogen ion concentrations and the averages calculated. These averages were then changed back to pH with the result that the mean was found to be 6.469 at cutting; 5.032 at 3 days of age; and 5.321 at 9 months of age.

Table 3 presents the average titratable acidity measurements at the various steps in the curd-making process.

Tables 2 and 3 indicate slight but noticeable differences in acidity between the three groups at the time of adding the rennet to the milk. These differences in both titratable acidity and pH measurements become increasingly evident as the cheese making process continues. There is a definite rise in pH in the interval between the addition of the rennet and immediately after coagulation of the curd. This increase is not gradual during this interval but apparently occurs at the moment of coagulation, since tests made immediately before and after visible coagulation showed increases in some instances as great as 0.15 pH. It is interesting to speculate on the

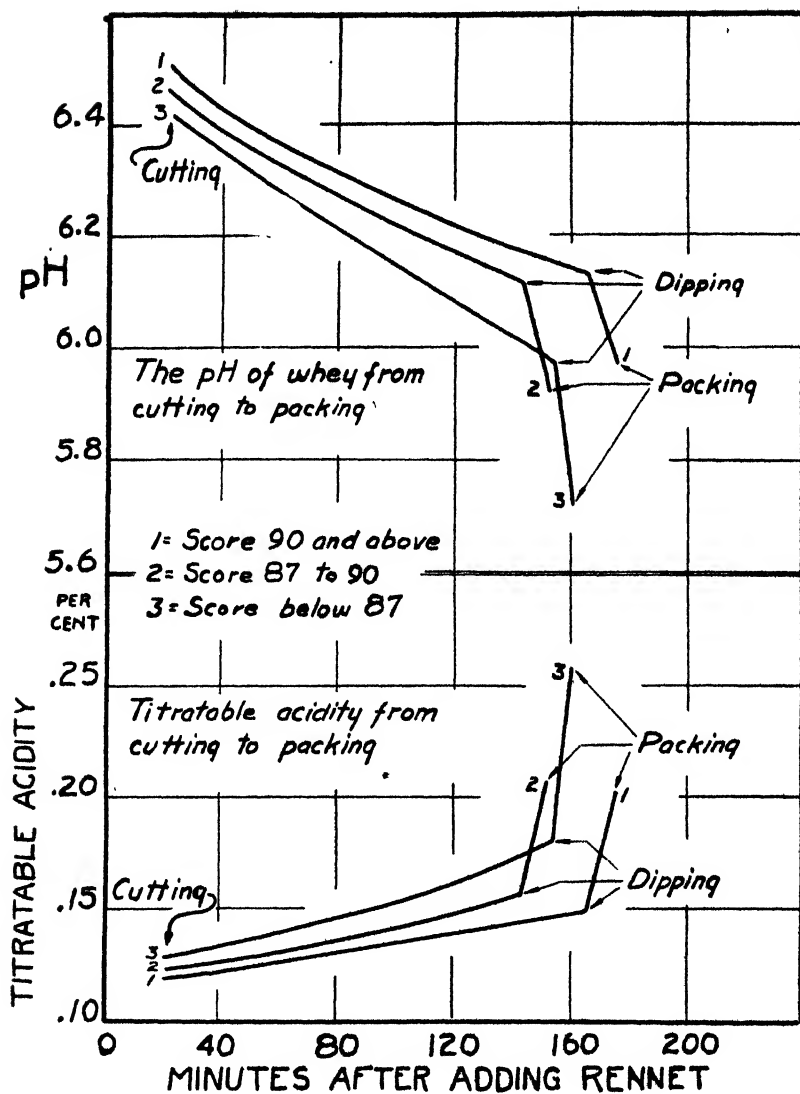


Figure 2

reason for this rise in pH but hardly within the scope of this paper to discuss it.

The average changes in acidity of the cheese in each group during curd-making are related to the time factor in figure 2. A significant difference between the three groups of cheese is illustrated in the angle of the break in each curve. The sharpest increase in acidity, after removing the whey, occurs in those lots of cheese in which the acidity develops most rapidly.

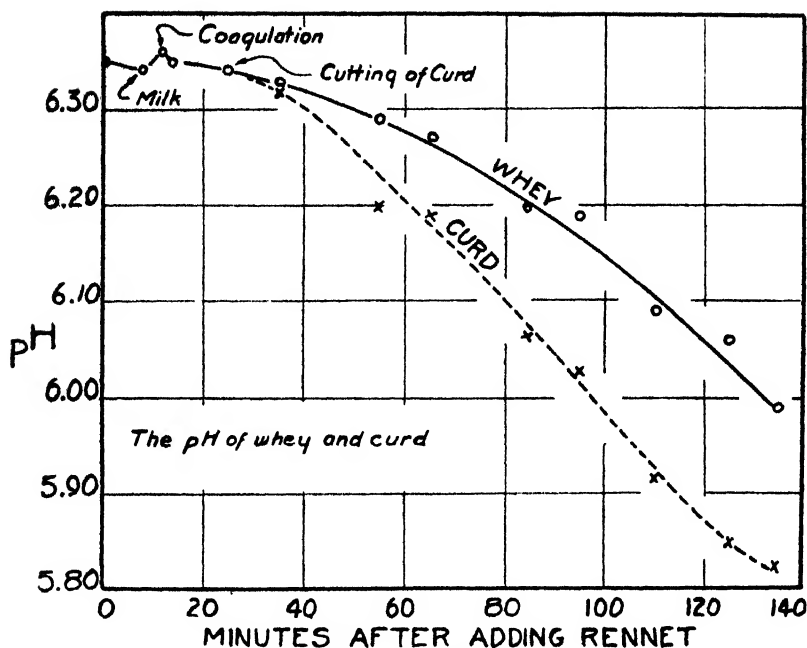


Figure 3

An explanation is suggested in the pH measurements of curd and whey from a batch of milk to which 5 per cent of starter was added. These measurements are plotted in figure 3.

It has been pointed out by Sammis, Suzuki, and Laabs (11) that the titratable acidity of the whey lags behind that of the curd. It is evident from figure 3 that pH measurements indicate the same trend. Other lots of cheese which showed less acid development in the making process before dipping were studied and it was found that as the rate of acid development decreases the difference between the pH of the whey and the curd decreases. It was also observed that after the bulk of the whey is removed from the curd that the pH of the small amount of whey which continues to drain from the curd approximates more closely, but not exactly, the pH of the curd. It was concluded that the changes in whey acidity between the intervals of dipping and packing, which are illustrated in figure 2, are indicative of the differences between the acidity of the whey and the curd which exist at the time of dipping and that these differences increase as the rate of acid development increases.

The relation between the acidity during curd-making and the quality of the cheese

The differences in acidity at the time of adding the rennet, as shown in tables 2 and 3, are not great enough to predict the quality of the cheese

which may result. This is true whether the acidity is measured as pH or by titration. At dipping and packing, however, the differences in acidity

TABLE 2
Average hydrogen ion concentration during the curd-making process

GROUP	I	II	III
Number of lots	14	25	15
Score of cheese	90 and over	87 to 90	below 87
	<i>pH</i>	<i>pH</i>	<i>pH</i>
Milk received*	6.56 ± 0.007	6.55 ± 0.006	6.55 ± 0.009
Rennet added*	6.47 ± 0.010	6.44 ± 0.007	6.40 ± 0.008
Cutting	6.51 ± 0.020	6.46 ± 0.007	6.42 ± 0.008
Heat on	6.43 ± 0.012	6.39 ± 0.007	6.37 ± 0.015
Heat off	6.35 ± 0.013	6.30 ± 0.009	6.26 ± 0.015
Whey removed	6.12 ± 0.016	6.12 ± 0.016	5.96 ± 0.031
Curd packed	5.97 ± 0.028	5.93 ± 0.021	5.74 ± 0.038
Curd milled*	5.29 ± 0.027	5.29 ± 0.014	5.12 ± 0.023
Curd pressed*	5.25 ± 0.019	5.20 ± 0.017	5.07 ± 0.020

* = measurements made on milk.

* = measurements made on curd.

All other measurements made on whey.

TABLE 3
Average titratable acidity during the curd-making process

GROUP	I	II	III
Number of lots	14	25	15
Score of cheese	90 and over	87 to 90	below 87
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Milk received	0.166 ± 0.0009	0.168 ± 0.0006	0.171 ± 0.0009
Rennet added	0.185 ± 0.0017	0.187 ± 0.0010	0.191 ± 0.0015
Cutting	0.122 ± 0.0015	0.125 ± 0.0007	0.129 ± 0.0014
Heat on	0.124 ± 0.0015	0.128 ± 0.0008	0.133 ± 0.0018
Heat off	0.131 ± 0.0020	0.135 ± 0.0010	0.142 ± 0.0025
Whey removed	0.151 ± 0.0031	0.157 ± 0.0024	0.181 ± 0.0040
Curd packed	0.201 ± 0.0056	0.210 ± 0.0054	0.259 ± 0.0105
Curd milled	0.556 ± 0.028	0.692 ± 0.028	0.781 ± 0.027
Curd pressed	No whey	No whey	No whey

between the three groups of cheese are considerably greater. This is more noticeable in figure 2 in which the acidity measurements are related to the time elapsing during the making operations. It is significant that the upper and lower halves of figure 2 are almost mirror images of each other. Regardless of the method of measurement, the highest scoring group of cheese is less acid at every stage of the making process, while the lowest scoring

group of cheese has the greatest acid development at every point of comparison.

It cannot be doubted from an examination of tables 2 and 3 and figure 2 that there is a definite relationship between the quality of the cheese and the acidity changes during the process of manufacture. A statistical study of the significance of the differences demonstrated by these pH and titratable acidity determinations indicates that the two methods of measurements are probably of the same relative value under the conditions of these experiments.

The relation between the pH and titratable acidity of the whey

In figure 4 the pH values of the individual samples of whey have been plotted against the corresponding titratable acidities. There is evidently a close correlation between these two methods of measuring acidity during the cheese-making process. Seventeen points beyond the range of the highest titratable acidity shown in figure 4, and 25 points which coincided with other observations between the pH limits 6.0 and 6.5, are not indicated, but they were used, however, in determining the slope of the original curve, of which figure 4 is a partial reproduction.

There is evidently a close correlation between these two methods of measuring acidity during the cheese-making process. It is not logical to believe, however, that the relationship which is implied by the curve in

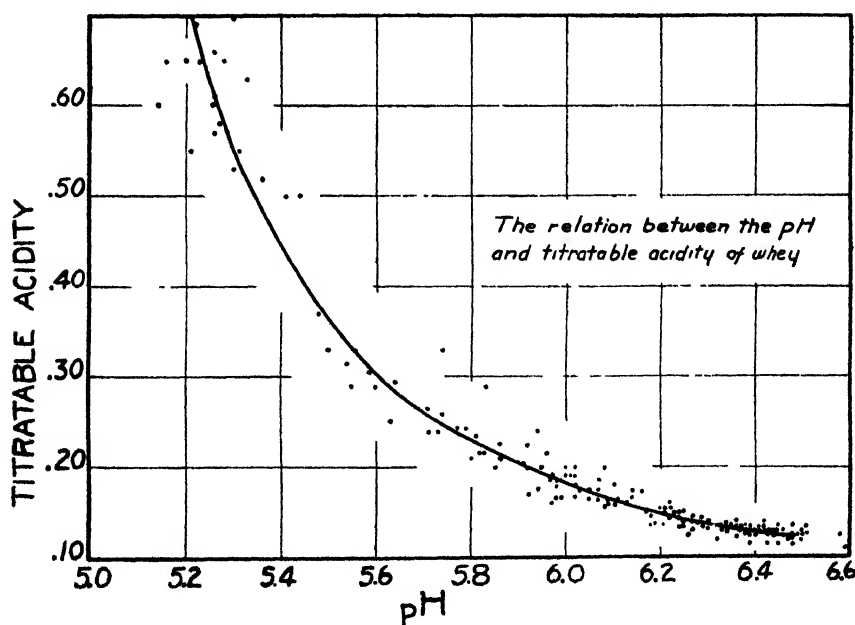


Figure 4

figure 4 would be constant under all conditions. Several factors might alter the slope of this curve if these experiments were duplicated. Three which should be mentioned are:

1. The types of organisms which dominated the acid development in the course of this study were probably characteristic of the local milk supply and of the starters which were used during the making experiments. Variations in these types might influence the identity of the products of fermentation and could logically affect the relation between the pH and the titratable acidity of the milk.

2. It has been demonstrated by Sharp and McInerney (1) that the initial acidity of fresh milk has a direct influence on the pH and titratable acidity measurements when that milk is allowed to sour. Cheese-making depends fundamentally on the development of acidity for the characteristic behavior of the curd. The initial acidity of the fresh milk undoubtedly influences the relation between these two methods of measuring acidity in the whey.

3. Studies of the buffer capacity of both individual and herd samples of milk (12, 13, 14 and 15) indicate the variable nature of this property. Obviously, variations in the buffer capacity would influence the nature of the curve shown in figure 4.

Acidity development in the cheese

The acidity measurements which were made during the ripening process of the cheese are summarized in table 4. These pH values show the same general trend in respect to quality as was exhibited in the cheese during the making process. The best cheese has the highest pH when fresh but the lowest pH after curing.

There are several fluctuations in acidity during the first week of curing which are difficult to explain. In general, however, there is a gradual increase in pH and increase in titratable acidity during the early stages of ripening. This is true for all three groups. It is interesting to observe that the cheese in the highest scoring group show the least change in pH during the period of these observations, while the cheese in the lowest scoring class show the greatest change in pH and finally actually attain a higher average than that developed by the better quality of cheese.

The pH values of the cheese in each group show a definite decrease during the first three or four days after making. One of the most significant features in this table is the regularity with which the best cheese maintains a pH above 5.0 during the entire ripening period. When the acidity of a cheese on the third day after it is made lays between 5.05 and 5.20, we have every reasonable assurance that it will not show acid defects at any later date.

TABLE 4
Average acidity during ripening

AGE OF CHEESE	GROUP I		GROUP II		GROUP III	
	Score 90 and above		Score 87 to 90		Score below 87	
	pH	Tit. Ac.	pH	Tit. Ac.	pH	Tit. Ac.
		%		%		%
1 day	5.12	0.70	5.08	0.79	4.94	0.84
2 days	5.07	0.80	5.03	0.86	4.86	0.93
3 days	5.05	0.82	5.02	0.87	4.90	0.94
4 days	5.03	0.80	5.00	0.89	4.88	1.01
5 days	5.06	0.85	5.03	0.87	4.89	1.02
6 days	5.05	0.85	5.02	0.91	4.89	1.04
7 days	5.06	0.87	5.03	0.93	4.92	1.07
14 days	5.03	0.98	5.06	0.97	4.94	1.16
21 days	5.07	0.96	5.06	0.97	4.97	1.19
35 days	5.12	1.05	5.11	1.01	4.97	1.21
49 days	5.13	1.05	5.11	1.02	4.96	1.25
9 months	5.32		5.41		5.33	
12 months	5.33		5.47		5.37	
24 months	5.58	1.19	5.76	1.20	5.76	1.27
Difference between ex- tremes	0.55	0.37	0.74	0.33	0.86	0.33

Unfortunately the titratable acidity measurements of the ripening cheese are not so dependable as are measurements of pH. The very nature of the method of analysis makes the results too variable. The degree of dispersion of the protein material which can be attained during the preparation of the sample for extraction varies decidedly in individual cheese during the first few weeks of curing. Those samples of cheese, which show the greatest tendency to dissolve in the warm water, carry through the filter the greatest amounts of protein material. The unsatisfactory relationship between the titratable acidity and pH measurements during the ripening of the cheese is more clearly shown in table 5, which presents the acidity measurements which were made on paired lots of cheese. Lots 82 and 83 were made from milk E, and lots 32 and 33 were made from milk K. The even numbered lots were made in a normal way to produce cheese of marketable quality. The other lots were treated in some manner to make high acid cheese. The results in table 5 illustrate the nature of the variations in the acidity measurements during the entire life of each cheese. They serve to emphasize the hopelessness of attempting to determine the quality of an unknown sample of cheese from a measurement of either pH or titratable acidity.

TABLE 5
The changes in pH and titratable acidity of individual cheese during making and ripening

MILK		B				K			
Cheese No.	82 (Score 90.8)		83 (Score 87.0)		32 (Score 87.3)		33 (Score 84.2)		
	<i>pH</i>	<i>Titr. ac.</i> %	<i>pH</i>	<i>Titr. ac.</i> %	<i>pH</i>	<i>Titr. ac.</i> %	<i>pH</i>	<i>Titr. ac.</i> %	
Receiving	6.53	0.170	6.53	0.170	6.42	0.165	6.42	0.165	
Setting	6.41	0.185	6.35	0.190	6.38	0.183	6.38	0.183	
Cutting	6.45	0.120	6.36	0.135	6.48	0.125	6.48	0.123	
Heat on	6.40	0.120	6.31	0.135	6.43	0.130	6.44	0.127	
Heat off	6.34	0.130	6.24	0.145	6.34	0.133	6.36	0.135	
Dipping	6.21	0.150	5.92	0.200	6.18	0.145	6.13	0.160	
Packing	6.08	0.200	5.74	0.330	5.98	0.185	5.94	0.240	
Milling		0.700		1.200	5.48	0.370	5.04	0.780	
Pressing	5.38		5.04		5.45		5.02		
1 day	5.09	0.70	4.92	0.86	5.28	0.68	4.93	0.85	
2 days	4.97	0.94	4.84	1.15	5.20	0.70	4.77	0.90	
3 days	4.99	0.94	4.83	1.08	5.19	0.68	4.81	1.04	
4 days	4.99	0.94	4.93	1.19	5.12	0.61	4.81	1.01	
5 days	5.07	1.04	4.97	1.15	5.12	0.72	4.88	1.08	
6 days	5.05	0.97	4.91	1.22	5.18	0.79	4.83	1.04	
7 days	5.06	1.04	4.93	1.24	5.11	0.72	4.82	0.94	
14 days	5.12	1.03	4.95	1.24	5.23	0.72	4.92	1.04	
21 days	5.20	1.04	4.95	1.15	5.18	0.65	4.90	1.19	
35 days	5.16	1.11	5.01	1.26	5.32	0.83	5.01	1.22	
49 days	5.12	1.06	5.01	1.21	5.19	0.76	4.94	1.33	
70 days	5.16	1.10	5.04	1.28					
9 months	5.28		5.16		5.45		5.42		
12 months	5.30		5.29		5.42		5.45		
24 months	5.58	1.12	5.69	1.22	5.72	1.17	6.01	1.15	

CONCLUSIONS

1. Electrometric measurements of hydrogen ion concentration in the whey during the manufacture of cheddar cheese, apparently do not indicate significant changes in the acidity any more accurately than the ordinary titration test. In the ripening process, however, these measurements can be easily duplicated and although they are not necessarily indicative of the quality of the cheese they might be used to indicate its suitability for some specific purpose, such as processing.

2. The quality of cheddar cheese is closely related to the acid development during the cheese-making process. The hydrogen ion concentration and titratable acidity are high at each critical point when the quality of the cheese is inferior.

REFERENCES CITED

- (1) SHARP, P. F., AND MCINERNEY, T. J. The relation of hydrogen ion concentration to the titratable acidity of milk. *Jour. of Biol. Chem.* 70: 729. 1926.
- (2) VAN SLYKE, L. L., AND BAKER, J. C. Free lactic acid in sour milk. *Jour. of Biol. Chem.* 35: 147. 1918.
- (3) VAN SLYKE, L. L., AND BAKER, J. C. Conditions causing variations in the reaction of freshly-drawn milk. *Jour. of Biol. Chem.* 40: 345. 1919.
- (4) RICE, F. E., AND MARKLEY, A. L. The relation of natural acidity in milk to composition and physical properties. *JOURNAL OF DAIRY SCIENCE* 7: 468. 1924.
- (5) KNUDSEN, S. Über die Messung der Wasserstoffionenkonzentration des Käses mittels der Chinhydronelektrode. *Zeit. Untersuch. Nahr. Genuss.* 50: 300. 1925.
- (6) WATSON, PAUL D. Use of quinhydrone electrode for following changes of pH in Swiss cheese. *Jour. of Ind. Eng. Chem.* 19: 1272. 1927.
- (7) WATSON, PAUL D. Personal correspondence. 1930.
- (8) SCHULZ, M. Über die Bedeutung der Wasserstoffionenkonzentration für die Beurteilung von Käse, insbes. Tilsiter Käse. *Molkerei Zeitung* 44: 527. 1930.
- (9) PASZTOR, S. Die Bestimmung der Wasserstoffionenkonzentration in Schmelzkäse mit Hilfe der Chinhydrone-Methode. *Molkerei Zeitung* 44: 1831. 1930.
- (10) Association of Official Agricultural Chemists. Official and Tentative methods of Analysis, p. 278, second edition, Washington, D. C. 1925.
- (11) SAMMIS, J. L., SUZUKI, S. K., AND LAABS, F. W. Factors controlling the moisture content of cheese curds. *Wisconsin Agricultural Station Research Bulletin* 7, 1910.
- (12) HOLM, G. E., WEBB, B. H., AND DEYSHER, F. F. The heat coagulation of milk. 1. Variations in the compositions, heat stability and other tests of milks from four cows during the course of a lactation period. *JOURNAL OF DAIRY SCIENCE* 15: 331. 1932.
- (13) BUCHANAN, J. H., AND PETERSEN, E. E. Buffers of milk and buffer value. *JOURNAL OF DAIRY SCIENCE* 10: 224. 1927.
- (14) WHITTIER, E. O. Buffer intensities of milk and milk constituents. 1. The buffer action of casein in milk. *Jr. Biol. Chem.* 83: 79. 1929.
- (15) HOLM, G. E., AND WEBB, B. H. Buffer capacities of various milks and proprietary infant foods. *Am. Jr. Dis. Children* 40: 260. 1930.

A STUDY OF FACTORS RELATED TO THE HARDENING OF ICE CREAM

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Recent studies conducted at this station, as well as elsewhere, have shown the importance of rapid freezing in the production of ice cream of smooth body. This applies not only to the ice formation that occurs in the ice cream freezer but that which takes place in the hardening room as well. Inasmuch as not more than half the water is usually frozen at the time the ice cream is drawn from the freezer, the hardening process becomes one of considerable importance. It was the purpose of this investigation to determine the relation of certain factors to the speed with which heat is removed from ice cream in a commercial type of hardening room.

PROCEDURE

Unflavored ice creams frozen in both batch and the Vogt continuous freezers were used. The hardening took place in a room 10 feet high, 8 feet wide, and 11 feet long, equipped with 1,150 feet of $1\frac{1}{4}$ inch expansion coils. Two adjacent rooms of this size were available.

All temperature readings were made by means of thermo-couples. This method is rapid, accurate to within one tenth of a degree, and adaptable for obtaining temperatures at any point in the product. The apparatus was placed on a table outside the hardening room which made it possible to check temperatures without entering the experimental area. The essential apparatus used was as follows:

1. Type K, Leeds and Northrup Potentiometer.
2. Type 2500, Leeds and Northrup Galvanometer.
3. Leeds and Northrup #30 D.C.C. Copper Thermo-couple wire.
4. Leeds and Northrup #30 D.C.C. Constantan Thermo-couple wire.

The thermo-couples were made by soft soldering the tips of the copper and constantan wire together. For protective purposes the tips were incased in slender paraffin filled glass tubes. The wires were coated with spar varnish so as to protect them against moisture. The thermo-couples were carefully calibrated against a Bureau of Standards thermometer. Readings were made every 30 minutes during the experimental period and unless otherwise specified all temperatures were taken at a central point in the container of ice cream.

FACTORS RELATED TO HEAT TRANSFER

When a difference in temperature exists between two adjacent bodies, there results a flow of heat toward the colder body. This transfer of heat

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may be due to either conduction, convection, radiation, or any combination of these three. An understanding of the characteristics of heat flow requires a general knowledge of these three types of heat propagation.

Conduction is a molecular transmission of heat, the material in question transmitting the heat from particle to particle of its own substance. If a flame is applied to one end of a copper rod, the heat passes toward the other end of the rod by conduction. The amount of heat transferred in this manner may be determined by use of the following equation:

$$Q = \frac{C A t_2 - t_1}{X} s$$

Q = quantity of heat in B.T.U.

C = coefficient of conduction.

A = area in square feet.

X = thickness in feet.

$t_2 - t_1$ = temperature difference between the two sections.

s = time in hours.

Convection denotes the transfer of heat energy within a liquid or gas by means of circulatory currents set up in the liquid or gas. The standard hot air heating system is an excellent example of this type of heat transmission. The rate of circulation depends principally upon the difference in density and viscosity of the hot and cold portions.

Radiation is the transmission of heat in space through an assumed medium commonly known as ether which is supposed to occupy all intermolecular space. The heat energy is supposedly converted into a wave motion of the ether and will thus even pass through clean dry air without a noticeable energy loss. All of the heat that the earth receives from the sun is in the form of radiant energy. The energy transmitted may be expressed as follows:

$$H = K(T_1^4 - T_2^4).$$

H = quantity of heat in B.T.U. per square foot.

K = the radiation constant.

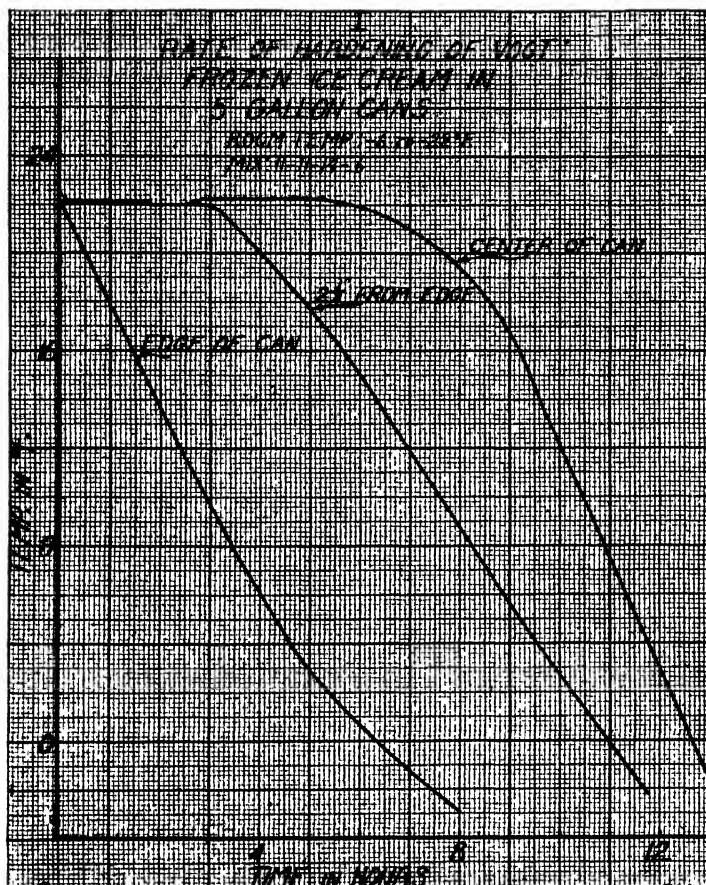
T_1 = absolute temperature of the radiating body.

T_2 = absolute temperature of the receiving body.

In the hardening room all of these three types of heat propagation are found but only conduction and convection are of major importance. The path of heat flow is naturally from the ice cream to the refrigerant which is usually confined ammonia. The heat which is derived from the latent and sensible heat of the ice cream will tend to flow through the cream and through the container walls much more rapidly than it can be removed from these walls by natural action. Therefore, the main problem is to accomplish the removal of heat from these walls as efficiently and rapidly as possible.

RATE OF TEMPERATURE CHANGE IN ICE CREAM WHILE HARDENING

To determine the rate of temperature change in a five-gallon metal can of ice cream during the hardening period the following experiment was performed. Thermo-couples were placed in the ice cream at the center of the can, $2\frac{1}{4}$ inches from the edge of the can and at the edge. All these junctions were 10 inches below the surface of the ice cream, which was one-half the depth of the can. Graph 1 shows the result of this study. In general,

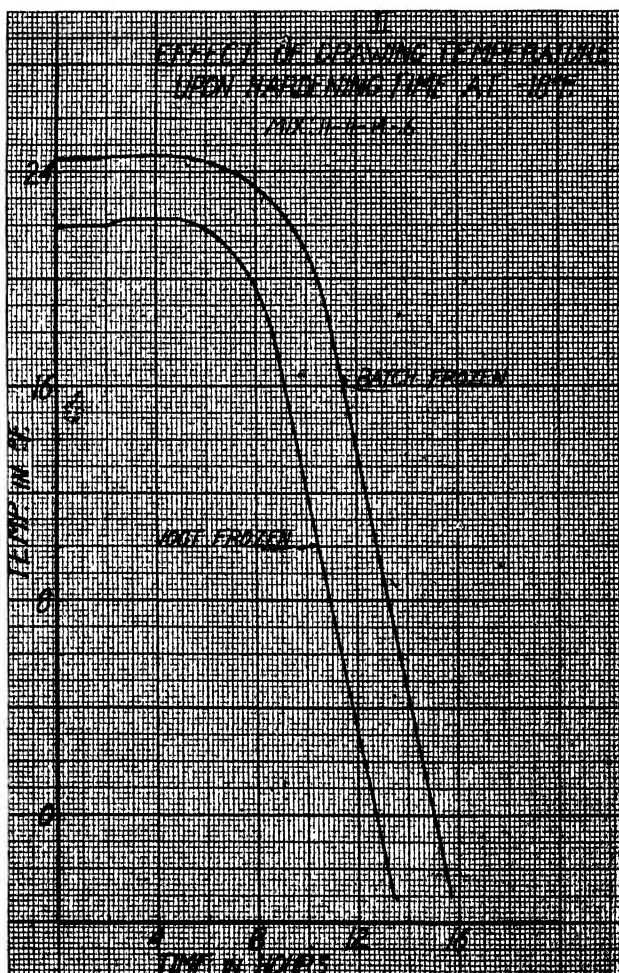


these data illustrate the insulating effect of the outer portion of the ice cream with regard to heat transfer from the center portion to the can walls. The temperature at the edge of the can started dropping immediately while that $2\frac{1}{4}$ inches from the edge did not start dropping appreciably until after three hours, while the portion in the center of the can remained at an almost constant temperature for approximately five hours. It will be noted that a slight increase ($.2^{\circ}$ F.) in the temperature of the

central portion occurred about three hours after the can had been placed in the hardening room and approximately two hours before the temperature curve started downward. This temperature rise was noted in practically all tests made in which the temperature was taken at the center of the can. The absence of a break in any of the temperature curves after they once started downward would indicate a cryohydric point in the ice cream is not reached within the temperature ranges studied.

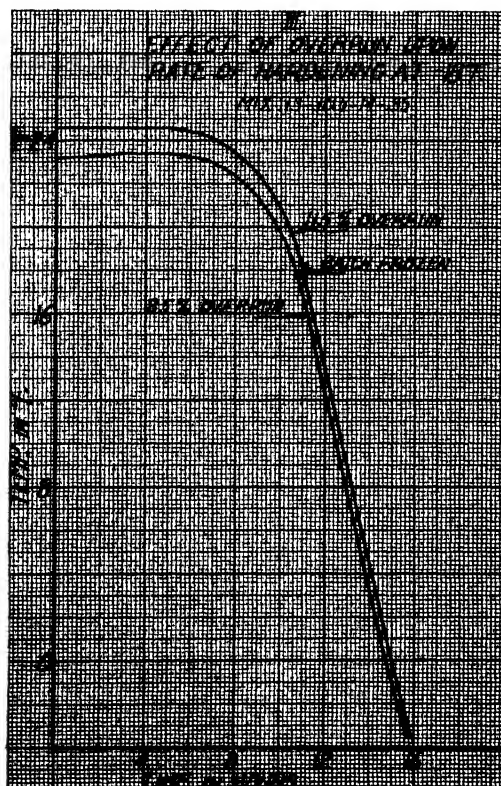
DRAWING TEMPERATURE

In general, the rate of hardening of ice cream depends upon the amount of heat to be removed and the rapidity with which the heat is conducted



away from the frozen product. The heat removed from the ice cream while in the hardening room consists of the sensible heat of the ice cream and the latent heat of the water that is frozen into ice during the hardening period. The percentage of unfrozen water is a function of the temperature of the ice cream. Therefore, the drawing temperature at the freezer is directly related to the percentage of water frozen and consequently to the time required to harden the ice cream.

By calorimetric methods it was determined that in the case of an ice cream mix containing 13 per cent fat, 11 per cent serum solids, 14.5 per cent cane sugar, 35 per cent of the water was frozen at 25°F ., 52 per cent was frozen at 22°F ., and approximately 82 per cent of the water was frozen at 0°F . It is evident, therefore, that the percentage of unfrozen water in the ice cream as it is drawn from the freezer has an important bearing upon the hardening time. That the drawing temperature is important is shown by graph 2 which shows the time required to harden ice cream in metal cans made from the same mix but frozen on different freezers. The batch frozen ice cream was drawn at a temperature of 24.5°F ., whereas the Vogt frozen product was drawn at 22°F . The curves



run practically parallel but that of the Vogt ice cream reaches 0° F. a little over two hours sooner. In other words, a difference of 2.5° at the beginning of the hardening period meant a saving of approximately 16 per cent in the time required to reach 0° F. When ice creams were drawn from the two freezers at the same temperature identical hardening curves were secured.

OVERRUN

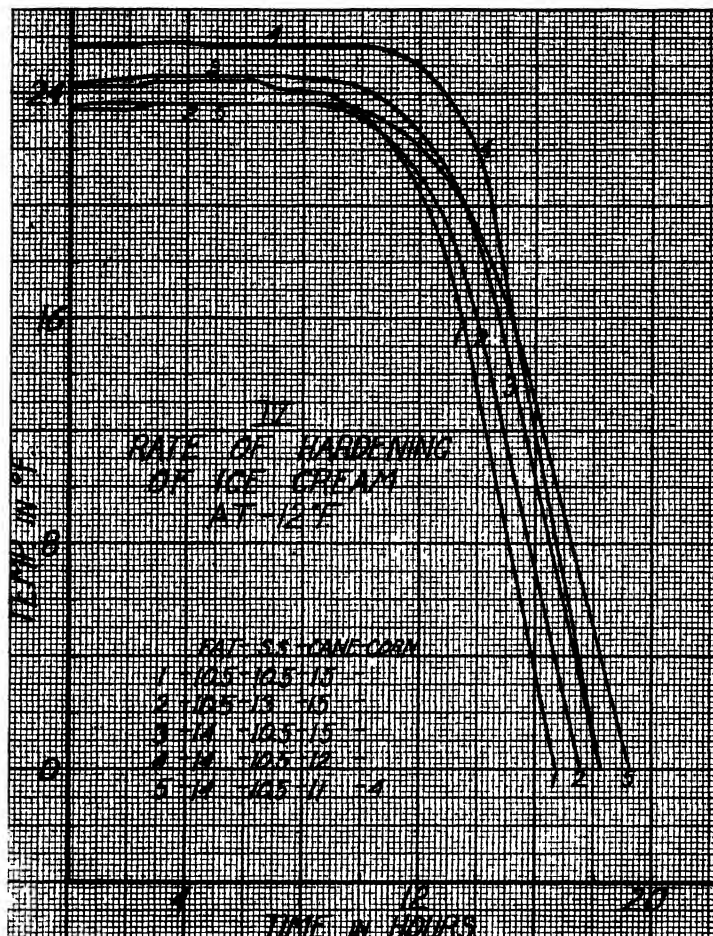
Graph 3 shows a comparison of the rate of hardening in metal cans of two five-gallon cans of the same ice cream differing only in overrun and consequently drawing temperature. It will be noted that although the 85 per cent overrun ice cream had a drawing temperature of 1.2° F. lower than that of the 115 per cent ice cream, the two reached zero at nearly the same time. Although the colder ice cream had a lower percentage of unfrozen water at the start, the greater amount of water that it contained per unit volume offset the effect of the temperature difference to a great extent. Since the hardening curves are much the same it may be concluded that the main effect of a change in overrun is a corresponding change in the number of heat units to be removed.

COMPOSITION OF ICE CREAM

Graph 4 shows the effect of variation in the milk solids and sugar content of the ice cream upon the rate of hardening. Variations in the soluble constituents of the ice cream resulted in differences in drawing temperatures due to the effect of these solids upon the freezing-point of the mix. It does not necessarily follow that ice creams drawn from the freezer at the same temperature will reach zero at the same time. This is shown by comparing the temperature curves of mixes 1 and 3, and 2 and 5. The rate of hardening increased as the initial freezing-point was raised as shown by comparing the temperature curves of mixes 3 and 4. Mix 5, which contained corn sugar and which had the lowest freezing-point had the slowest rate of hardening. This difference in the rate of hardening is directly traceable to the amount of unfrozen water. As the freezing-point is lowered the amount of water to be frozen between the temperature range of 20° and 0° F. increases which results in a decreased rate of fall in the ice cream temperature curve.

RAPID HARDENING

During the past few years rather extensive use has been made of electric fans specially made for hardening room operation. These fans have been found to shorten the hardening time which is an advantage not only from the standpoint of improved texture but also because of saving in time required to make the ice cream sufficiently hard to market. The fan used in this study was one supplied by the Meir Electric Company, of Indianapolis.

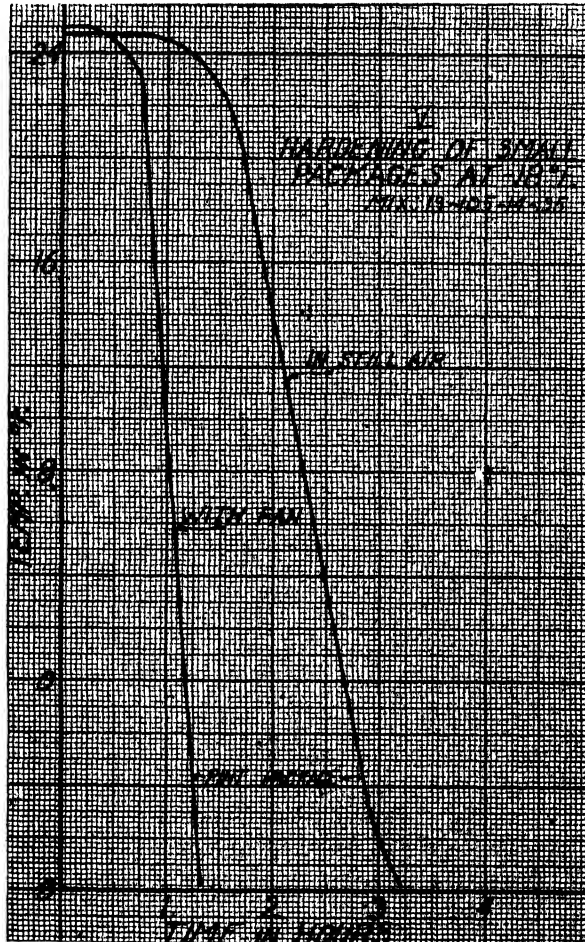


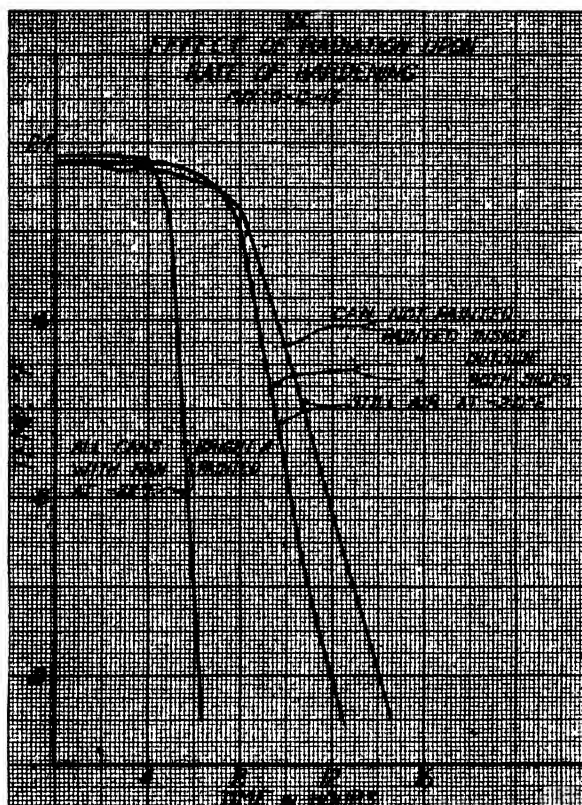
It was 24 inches in diameter and had an air velocity of 1,500 linear feet per minute. Hardening rooms identical in size and construction were used in comparing the rate of hardening ice cream with and without the use of an electric fan.

Heat passes from the ice cream container walls to the surrounding cold air by conduction and this air, becoming somewhat warmer, tends to rise thus carrying the heat away from the ice cream cans or packages. However, there is necessarily a thin film of stagnant air between the can wall and the moving air. As air is a very poor conductor of heat, this dead air film acts as an excellent insulator for the container. Therefore, the thickness of this air film to a great extent controls the rate of hardening. The conductivity coefficient of steel is 26.2 B.T.U. $\frac{\text{ft.}^2 \times ^\circ\text{F.} \times \text{hr.}}{\text{ft.}}$, while that of air is approximately 0.0129. Using these figures it is easily determined

that if an air film of 0.01 of an inch thickness were to be placed by a steel wall of equal resistance to heat flow, this steel wall would be about 20 inches thick. Data from "Mark's Handbook" give the conductance of an air film (based upon quiet air and a 50° F. temperature differential) as 1.95 B.T.U. per square foot per $^{\circ}$ F. per hour. It is apparent, therefore, that the thickness or construction of the metal can wall is of minor importance in this type of heat transmission.

However, by the use of forced convection currents, such as those set up by a blast of air from a fan, the air film surrounding the container wall is partially swept away, thereby decreasing the hardening time. This saving in hardening time amounted to 50 per cent in the case of five-gallon cans. For smaller packages, an even greater reduction was made in the time required to harden to 0° F. Graphs 5 and 6 illustrate this point clearly.

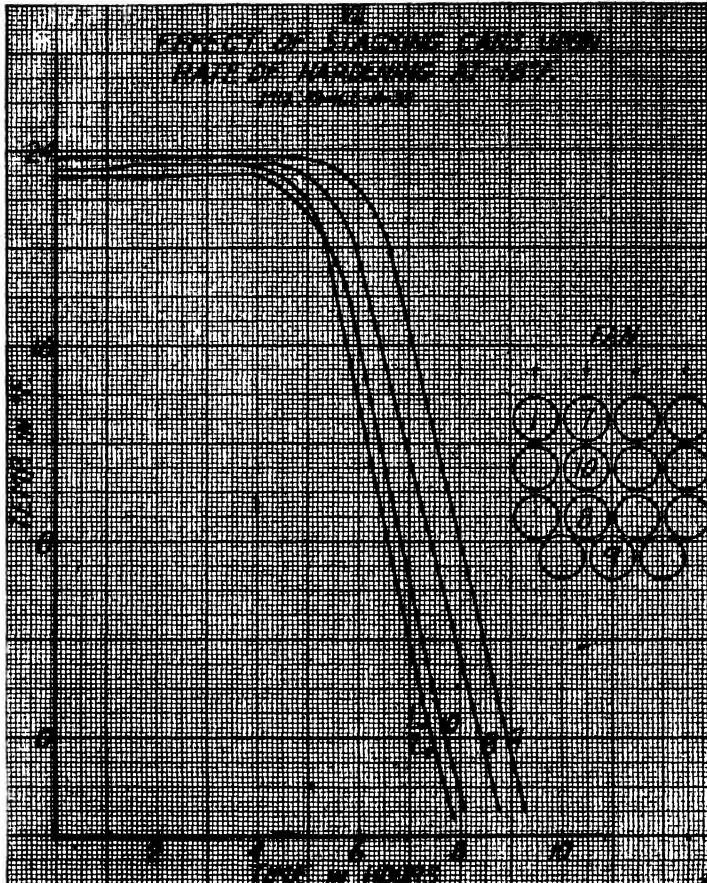




These comparative tests were made on identical samples placed in rooms of identical size and construction connected with the same compressor.

It might be added that the same type of resistance to heat transmission exists in the case of the heat passing from the air through the refrigerant piping. The air film around the pipes is also swept away to a great extent by the air blast thereby reducing the resistance. This action alone will enable the compressors to cool the room much more efficiently, due to the decreased temperature differential between the refrigerant and the circulating air. Observations made during hardening tests show that with a fan in operation, the same amount of heat can be removed per hour with a higher absolute back pressure. This will reduce power costs as well as increase the capacity of the compressors.

That the relative position of the fan and ice cream can is important is evidenced by graph 7 which shows the effect of stacked metal cans upon the rate of hardening. These cans were placed as close together as possible and a fan was placed about three feet in front of the first row of cans. The air velocity was decreased tremendously by the obstruction but hardening time under the condition of the experiment was not seriously affected. In the



cooling of stacked cans, the best efficiency will be obtained from the air blast by having the cans as much in the open as possible.

HEAT RADIATION FROM ICE CREAM CANS

So far no consideration has been given to radiation as a factor in the hardening room. From the definition of radiation, the cans may be expected to pass off some heat as radiant energy. Hardening tests upon five-gallon metal cans, standing alone in still air, have shown that ice cream cans painted on the outside with other than metallic paints will harden in about 85 per cent of the time required for ice cream in similar cans with bright surfaces. This is shown by graph 6. Painting the inside of the can was of no benefit. This is entirely in line with the theory of radiation, these data checking closely with reliable tests conducted upon steam and hot water radiators.¹ It is apparent therefore that ice cream cans painted

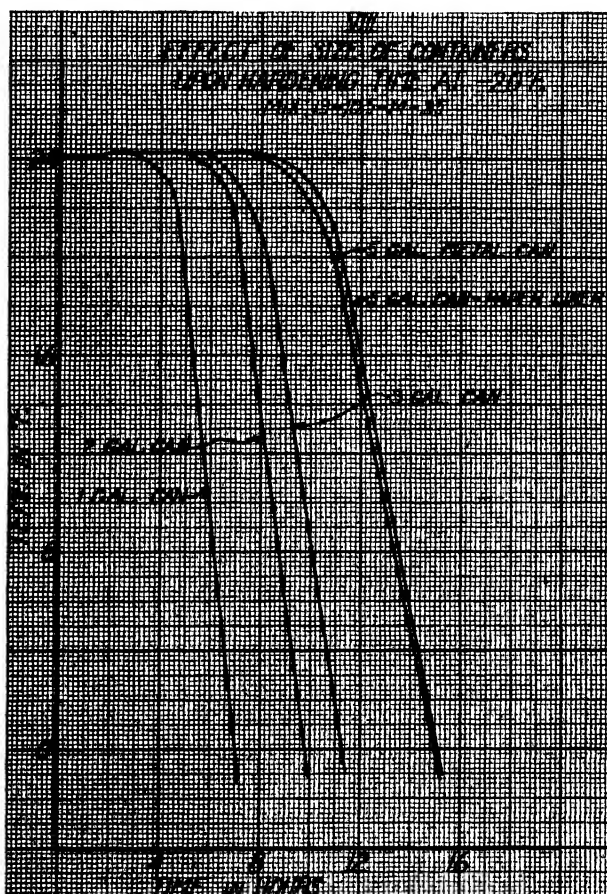
¹ Harding and Willard. *Mechanical Equipment of Buildings*. Vol. 1, p. 274.

on the exterior will be nearly perfect radiators while the opposite will be true of unpainted cans.

However, this difference in hardening time due to radiation may not be attained in actual hardening room practise. If cans are stacked in groups, those that are enclosed upon all sides will receive as much energy as they send out, thereby reducing the effect of heat transmission in this manner to practically zero. Furthermore, if a fan is used, the total radiant energy emitted is greatly reduced. This is due to a decrease in hardening time and to a much lower surface temperature. The data below are from actual tests upon ice cream under practical conditions.

Temperatures - °F.

	Room	Can Wall	Center of 5-gal. Can
Natural convection	- 17.4	- 5.6	19.3
Forced " "	- 18.3	- 14.5	19.0



Substituting these known values in the formula for the radiant energy transmitted, $H = K(T_1^4 - T_2^4)$, in the case of natural convection (N) and forced convection (F) the following values are obtained—

$$\begin{aligned} H_N &= K(454.2^4 - 442.4^4) = 10580 = 3.1 \\ H_F &= K(445.3^4 - 441.5^4) = 3370 = 1 \end{aligned}$$

It is evident, therefore, that under the conditions of the experiment the lower surface temperature noted under forced convection would reduce the amount of heat emitted by radiation at the can wall by about two-thirds.

As shown by the data in graph 6, in the case where forced convection was used there was no difference in the hardening time of the ice cream in the painted and unpainted cans. The unpainted can, however, contained ice cream which had a slightly higher initial temperature which probably accounts for the two curves coinciding in the lower temperature range, otherwise there would have been a slight difference in favor of the can painted black.

TYPE OF PACKAGE

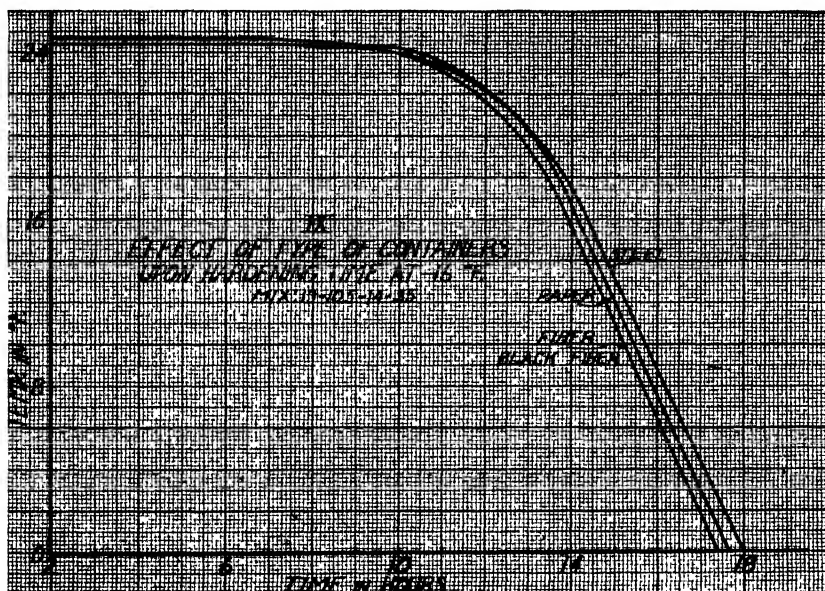
The hardening time for various size metal containers is shown in graph 8. The curve for five-gallon cans with paper liners has been closely checked and the maximum increase in hardening time was found to be under 2 per cent. Tests upon these containers and numerous packages have shown the hardening time to be a function of the cross sectional area and shape of the package. The data given below are for small paper packages hardened in still air at approximately -18°F .

Shape of Package	Dimensions in Inches of Package	Paraffined	Initial Temp. of Ice Cream Degrees F.	Time to Harden to 0°F . in hrs.
Cylindrical	$3\frac{1}{2}$ dia. \times $6\frac{1}{2}$ long	Yes	24.3	4.65
	$3\frac{1}{2}$ " \times $6\frac{1}{2}$ "	No	24.3	4.60
	$3\frac{1}{2}$ " \times 3 "	No	24.4	3.90
	5 " \times $4\frac{1}{2}$ "	Yes	24.0	5.50
Rectangular	$2 \times 3\frac{1}{2} \times 7\frac{1}{2}$	Yes	24.6	3.95
	$3 \times 4\frac{1}{2} \times 5$	Yes	24.1	4.35
	$2\frac{1}{2} \times 3\frac{1}{2} \times 6\frac{1}{2}$	Yes	24.8	4.60
Tapering	$3\frac{1}{2} - 2\frac{1}{2}$ dia. \times $4\frac{1}{2}$	Yes	23.9	2.85
	$4\frac{1}{2} - 3\frac{1}{2}$ " \times $2\frac{1}{2}$	Yes	24.1	3.25
	$4\frac{1}{2} - 3\frac{1}{2}$ " \times 3	Yes	24.1	3.50
	$5\frac{1}{2} - 4$ " \times 4	Yes	24.1	4.70

It is distinctly noticeable that the paraffining of paper packages did not decrease the rate of hardening. Paraffin undoubtedly will add to the re-

sistance to heat flow through the package wall but this addition is negligible when compared to the total resistance to the passage of heat.

In recent years, the industry has made extensive application of single service paper containers for marketing ice cream. Tests were made to compare the rate of hardening of ice cream in 20-quart containers of paper, fiber, and steel construction. The paper cans were laminated and were covered on the outside with sulphate paper and were lined on the inside with paraffin treated bond paper. The fiber cans were formed from wood fiber and were impregnated with water proofing material. The steel cans were of standard design with a bright tinned surface. It might be expected that the insulating effect of the paper would lengthen the hardening period, however, graph 9 shows that five-gallon paper or fiber cans standing alone



will harden faster than a five-gallon steel can under like conditions. A fiber can painted black showed no change in hardening time as compared to the unpainted can. This shows the fiber can to be a first-class radiator. However, in the case of stacked cans, where radiation is a negligible factor, the steel cans would probably show to better advantage in hardening time.

SUMMARY AND CONCLUSIONS

By means of thermo-couples accurate within 0.1° F., temperature changes occurring during the hardening of ice cream were studied. It was determined that in still air the temperature of the center portion of a five-gallon can of ice cream remained constant for about 5 hours. The

temperature then fell fairly rapidly, reaching zero after about 13 hours in a -18° F. hardening room.

The drawing temperature of the ice cream is directly related to the percentage of water frozen and consequently the hardening time. A difference of $2\frac{1}{2}^{\circ}$ in drawing temperature resulted in a saving of approximately 16 per cent (about $2\frac{1}{2}$ hours) in the time required to reach zero.

Variations in overrun result in differences in drawing temperatures and amount of water per unit volume to be frozen. There was little difference in the hardening time of ice creams containing 85 and 115 per cent overrun due to the balancing effect of differences in initial temperature and amount of water present per unit volume.

As the freezing-point of the mix was raised the rate of hardening was increased. This was particularly true between 22° and 0° F. This was likely due to differences in the amount of water to be frozen in this temperature range, the high freezing point mixes having relatively more of the water frozen upon reaching 22° F.

By means of an electric fan placed in the hardening room the rate of hardening was increased approximately 100 per cent. This was thought to be due to the effect of the convection currents sweeping away the air film surrounding the ice cream container.

By painting the exterior surface of ice cream cans black it was possible to reduce the hardening time in still air about 15 per cent due to the greater heat radiation from the darkened surface. When hardened in the presence of forced convection currents the amount of heat transferred by radiation was greatly reduced due to a shorter hardening period and a lower temperature at the surface of the container.

Tests upon various types and sizes of ice cream packages show that the hardening time is dependent upon the area and shape of the package. Paraffining of paper packages did not decrease the rate of hardening.

Paper liners in five-gallon cans increased the time of hardening less than 2 per cent. Ice cream hardened faster in paper cans than in steel cans. Fiber cans conducted the heat from the ice cream more rapidly than either paper or steel cans. Painting fiber cans black did not alter the rate of heat transfer indicating the fiber surface to be a good radiator.

American Dairy Science Association Announcements

OFFICERS OF SECTIONS AND DIVISIONS

The newly elected officers of Sections and Divisions are given on the directory page as reported to the Editor.

EASTERN DIVISION MEETING

The twelfth annual meeting of the Eastern Division of the American Dairy Science Association was held September 17-19, 1933, at the Hotel Clinton, Springfield, Massachusetts. Attendance at the business meeting was 28 with chairman C. B. Bender, presiding. Due to conflicting meetings in Chicago the following day's program was poorly attended but 67 attended the banquet to hear Dr. J. G. Lipman speak on stabilizing the dairy industry.

The program included talks on artificially dried young grasses by J. A. Newlander, sanitary milk production by J. D. Brew, pasture grasses and clovers by R. C. Foley, succulence in the ration by G. C. White, vitamin A content of milk by W. C. Russell, the course in market milk by H. G. Lindquist, mastitis by J. G. Huecker, and mastitis and soft curd milk by R. C. Welch and F. J. Doan.

R. W. Smith, Jr., reported that in the Dairy Products Judging Contest Vermont was first, Massachusetts second, and Connecticut was third. Five teams competed. In the Dairy Cattle Judging Contest, A. R. Merrill reported that Maryland was first, Rutgers was second, and Cornell was third. In the judging of General Livestock, E. N. Boland reported that Pennsylvania was first, Massachusetts was second, and New Hampshire was third.

R. E. JOHNSON, *Secretary*.

JOURNAL OF DAIRY SCIENCE

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THE GROWTH OF *PENICILLIUM ROQUEFORTI* ON SYNTHETIC MEDIA

N. S. GOLDING

Washington Agricultural Experiment Station, Pullman

INTRODUCTION

The manufacture and ripening of blue veined types of cheese, such as Roquefort, Stilton and Wensleydale, present many difficulties if a high quality of cheese is to be made (18) (4) (21). Thom and Currie (22) show that the dominance of Roquefort mold in cheese is dependent upon a relatively narrow margin of oxygen which gives the right air supply. With Wensleydale cheese made under relatively well controlled conditions, Golding (10) has shown that the development of the mold is a matter of considerable uncertainty.

The process of manufacture and ripening of blue veined cheese may well be said to be an art and standardized methods cannot be adopted due to the existence of too many variables.

With these considerations in view, it was realized that another and entirely different approach to mold ripened cheese might be possible. The pasteurization or processing of cheese has made particularly rapid strides in the last few years (19). Therefore, where it is possible to develop a satisfactory enzyme concentrate this might be added to the cheese subsequent to heating and the desired flavor develop in the processed cheese.

In considering the above idea, it was appreciated that at first further knowledge must be obtained as to the growth of the mold and conditions that will produce maximum growth in a suitable medium.

Naylor *et al.* (15), working with *P. roqueforti* (Thom) have shown that the largest mold growth obtained produced the greatest enzyme formation. Knapp (14) shows that mold growth and enzyme formation go hand in hand. Further the quantity of a particular enzyme produced is dependent on the medium on which the mold is grown (7) (20) (13).

The particular salts required by the molds when growing in a synthetic medium are those in Czapeks formula modified by A. W. Dox (22) (11).

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That such salts are satisfactory for the growth of *P. roqueforti* has also been shown (15) (11). However, Naylor *et al.* (15) have obtained considerable advantage in growth by replacing NaNO_3 by NH_4Cl and by reducing the quantity of NH_4Cl used when casein is included in the medium.

For the organic nutrients of the medium casein and dextrose have been shown to be suitable (11) (15). On the other hand, lactose is not as satisfactory (11).

The optimum temperature of growth is of importance as shown by Knapp (14). The optimum temperature of growth for the culture of *P. roqueforti* used in these experiments has been shown to be between 20.6°C . and 28.9°C . (11).

The initial reaction of the media for the growth of *P. roqueforti* was considered by Naylor *et al.* (15). The change in reaction of media produced by the growth of microorganisms has been investigated (12) (3) (6) (8) (1). In the presence of sugar the reaction is changed to the acid side (3) (6) (8) (1) and later to the alkaline side (3) (6) (1).

The wide range of pH possible during growth of mold as shown by Johnson (12) presents a problem as to the type of proteolytic enzymes likely to be elaborated. Buchanan and Fulmer (5) show pepsinases are most active at pH 2.0 to 5.0, tryptases at pH 7.0 to 9.0. Dox (7) working with *P. camemberti* attributes the casein digestion as due to a type of "erepsin," and is of the opinion that plant ereptase has its reaction range in the direction of acidity. However, in his summary of the literature Dox (7) states: "The nature of the protease has not been established, though most of the authors consider it as closely resembling trypsin."

As to whether the protease elaborated by molds is extra or intracellular would appear to be limited by the stage of growth as the greatest enzyme action is found in the mold filtrate when sporulation is well advanced (7) (15). The secretion of enzymes into the culture medium does not ordinarily occur to any appreciable extent during the active vegetative period of the mold. However, Buchanan and Fulmer (5) are of the opinion that the reaction of the medium may be important in determining whether an enzyme will pass the filter.

MATERIALS AND METHODS

Culture of Mold. In all the work done in this investigation a culture of *P. roqueforti* designated No. 32 was used. This culture is described under No. 16 in (11) and its casein digestion is given in (10) under No. 32. Since isolation, the culture has been retained in our mold culture collection on potato or wort agar. Transfers have been made twice each year.

Growth for Inoculation. When required for any experiments fresh cultures were grown on potato or wort agar and the inoculum obtained from the well developed slant into 100 ml. of distilled water. One ml. of this water was used to inoculate each flask.

MEDIA

Salts. The salts used in preparing all the synthetic media for each 1000 ml. of final medium were:

MgSO ₄50 gram
K ₂ HPO ₄	1.00 "
KCl50 "
FeSO ₄01 "
NH ₄ Cl (0.03 N)	1.61 "

These salts in the above proportions were used by Naylor *et al.* (15).

Butterfat. The butterfat when used was obtained from fresh, sweet cream butter. This fat was emulsified into the medium by the use of a small hand power Hurcol emulsifier.

Casein. Will Corporation (Rochester, N. Y.) acid casein was used in all the experiments and was obtained in solution by the method adapted by S. Henry Ayres (2), the amount of NaOH and HCl being varied according to the percentage of casein used. The final reaction of the medium was adjusted to a pH between 6.20 and 6.00.

Dextrose. Difco bacto dextrose was used in the proportions as stated, being added to the medium before it was made up to volume.

CaCO₃. Where CaCO₃ was used 0.2 grams of precipitated chalk were weighed separately into each flask.

Media Flasks. The medium was added in measured quantities of 25 ml. to each 125 ml. Erlenmeyer flask, which was then plugged and sterilized at 12 pounds pressure for 20 minutes. For the nitrogen determination each flask was weighed to the second decimal place so that the loss by evaporation could be made up before filtering.

Hydrogen-ion Determination. All pH determinations were made at 25° C. using quinhydrone, a gold electrode and a Leeds and Northrup potentiometer No. 7654. The determinations in every case were made on the undiluted filtrate.

Quantitative Determinations for Sugar. The quantitative determinations for dextrose were made with Fehling's solution.

In several cases where considerable sugar is recorded by the above method quantitative determinations by the Schaffer Hartmann method did not show more than 0.3 per cent. When using a medium of 15 to 20 per cent dextrose as much as 0.3 per cent might come from the part which unavoidably dries on the inside of the flask and, therefore, cannot be reached by the mold.

Felt Weight. The felt from each flask, at the required age, was filtered off into weighed Gooch crucibles. The filtrate was collected for pH, sugar, and nitrogen determinations. The felts were washed with distilled water, dried at 100° C. till constant weight. The weight of dry matter does not

express the exact weight of mycelium due to some precipitate being thrown down in sterilization and also to the precipitation of the casein by the acid formed. However, these weights are comparable and when expressed with the pH of the filtrate a satisfactory interpretation can be made.

Nitrogen Distribution in Filtrate. The 125 ml. Erlenmeyer flasks, both the control and those in which the mold had grown, were made up to weight with distilled water and filtered. Sufficient flasks were taken to provide about 230 ml. of the required filtrate. The total nitrogen of the filtrate was determined on 5 ml. lots in triplicate. The protein breakdown was determined according to the methods described by Eagles and Sadler (9) for the following:

1. The soluble nitrogen (acetic acid filtrate).
2. The amino nitrogen (phospho-tungstic filtrate).
3. The proteose nitrogen (sodium sulphate precipitate)
4. The peptone nitrogen (tannic acid precipitate).
5. The sub-peptone nitrogen (tannic acid filtrate).

One and two were adapted from the methods of Orla-Jensen (16) (17) and three, four and five from the methods of Wasteneys and Borsook (23).

EXPERIMENTAL

The Growth of P. roqueforti on the Standard Salt Medium Plus Two Per Cent Butterfat and One Per Cent Casein and Various Percentages of Dextrose

The 125 ml. flasks containing 25 ml. of the above medium with nil, 2.5, 3.0, 3.5, 4.0, 4.5, and 5.0 per cent of dextrose respectively were inoculated and grown for a period of ten days at 23° C.

The results obtained, given in table I, show:

1. The weight of dry felt produced (whether extracted with ether or not) increases with the increased percentage of dextrose in the medium.
2. With nil and 2.5 per cent dextrose the filtrate had been changed towards the alkaline side. In all other flasks the reaction had been changed more to the acid side.
3. The greater the percentage of dextrose in the medium the higher the acidity produced in the filtrate.

The change in reaction of the filtrate due to growth is given on the 4, 6, 8 and 10th days respectively. From these determinations it is seen that there is a close relationship between the presence of dextrose and the reaction of the filtrate. As soon as the dextrose is used up by the growth of the *P. roqueforti* the reaction of the filtrate gradually moves towards the neutral. No acidity is produced in the medium without dextrose.

The Growth of P. roqueforti in the Standard Salt Medium Plus Various Percentages of Casein and Dextrose

The standard 125 ml. flasks containing 25 ml. of the medium, 1 per cent casein and 2.5, 3.0, 4.0, 5.0, 6.0 and 7.0 per cent of dextrose respectively together with flasks of 2 per cent casein and 8 and 10 per cent of dextrose respectively were inoculated and incubated at 22° C.

The weight of dry felt, the pH of the filtrate and the presence or absence of sugar are given in table II which shows:

1. The amount of growth of *P. roqueforti*, as expressed by the weight of felt, is dependent upon the percentage of casein and dextrose. Further an excess of dextrose over casein, beyond a certain point (about in the ratio of 5 to 1) does not materially increase the growth.

2. The maximum felt weight is obtained during the growth of *P. roqueforti* in the medium when all the sugar is used and from then on the weight of felt decreases.

3. The proportion of dextrose to casein is an important factor in determining the reaction of the filtrate during the growth of *P. roqueforti*.

4. As soon as the dextrose is used up by the mold the reaction of the filtrate tends to approach neutral.

5. In the case of the medium, containing 1 per cent casein and 7 per cent dextrose, the casein would appear to be insufficient to allow the growth of mold to use up all the dextrose.

In the case of the 2 per cent casein and 8 per cent dextrose, the difference is striking; here the dextrose is almost all used up by the 12th day and the filtrate is changed towards neutral.

The Growth of P. roqueforti on the Standard Salt Medium with High Percentage of Casein and Dextrose

The object of this experiment was to determine how far increased growth as measured by weight of felt could be obtained by increasing the percentage of casein and dextrose in the medium in the right proportion. Erlenmeyer flasks of 125 ml. with 25 ml. of the medium containing the same standard salts were prepared with various percentages of casein and dextrose (see table III). These were inoculated with *P. roqueforti* and incubated at 20° C. with a high relative humidity in the incubator.

The pH of the filtrate, the weights of dry felt and the presence or absence of sugar are given in table III.

1. The growth as shown by the weight of felt increases with the percentage of casein and dextrose.

2. The time required to obtain the maximum felt weights is in proportion to the percentage of nutrients. A reduction in the weight of felt after the 14th day with mold growing in media of 10 to 13 per cent dextrose and growth increase with the remaining flasks of higher concentration until the 17th day. After the 17th day all felts show a reduction in weight.

TABLE I
Growth of *P. roqueforti* (Culture 32) on Standard Salt Medium + 2% Butter Fat; 1% Casein and Various % Dextrose

Inoculated October 18, 1929									
Incubated at 23° C.									
DAYS GROWTH	4		6		8		10		
	pH	Sugar	pH	Sugar	pH	Sugar	pH	Sugar	Weight of dry ex-tracted felt
Nil	6.05	Nil	6.80	Nil	7.05	Nil	7.25	Nil	.21
2.5	3.40	Present	6.10	Nil	6.70	Nil	6.75	Nil	.47
3.0	3.40	Present	4.70	Nil	6.35	Nil	5.50	Nil	.56
3.5	3.30	Present	3.45	Trace	5.80	Nil	5.55	Nil	.56
4.0	3.20	Present	2.55	Trace	4.20	Nil	5.35	Nil	.56
4.5	3.00	Present	2.55	Trace	2.85	Trace	3.70	Nil	.57
5.0	3.05	Present	2.25	Present	2.50	Present	2.20	Nil	.63

TABLE II
Growth of *P. roqueforti* (Culture 32) in Standard Salt Medium + Various % of Casein and Dextrose

Incubated at 22° C.			Inoculated November 22, 1929									
DAYS GROWTH			10		12		14		16		18	
% Casein	% Dextrose		pH	Weight of dry felt	Sugar	pH	Weight of dry felt	Sugar	pH	Weight of dry felt	Sugar	Weight of dry felt
1	2.50		7.70	.34	Nil	7.85	.32	Nil	7.90	.31	Nil	
1	3.00		7.40	.40	Nil	7.70	.38	Nil	7.85	.37	Nil	
1	4.00		6.30	.51	Nil	7.25	.47	Nil	7.25	.43	Nil	
1	5.00		2.90	.62	Trace	2.65	.57	Nil	2.50	.53	Trace	
1	6.00		2.25	.62	Present	2.05	.60	Present	2.25	.64	Trace	
1	7.00		2.05	.63	Present	2.05	.62	Present	2.10	.68	Present	
2	8.00		3.80	.86	Present	5.45	.89	Trace	6.70	.89	Nil	.79
2	10.00		3.15	.92	Present	3.70	1.10	Present	5.75	1.10	Nil	1.03

TABLE III
Growth of P. raoultii (Culture 32) on Standard Salt Medium with high Percentages of Casein and Dextrose

Incubated at 20° C				Inoculated December 20, 1929						
DAYS GROWTH		14 DAYS			17 DAYS			20 DAYS		
Casein %	Percentage	pH	Weight of dry felt	Sugar	pH	Weight of dry felt	Sugar	pH	Weight of dry felt	Sugar
2	10	5.25	1.09	Slight	6.00	1.09	Trace	6.70	1.01	Nil
3	11	6.45	1.30	Consid- erable	7.35	1.25	Trace	7.55	1.20	Nil
3	12	6.80	1.37	Slight	7.25	1.36	Trace	7.35	1.34	Trace
3	13	6.30	1.49	Heavy	7.15	1.38	Trace	6.85	1.31	Slight
3	14	5.05	1.44	Very heavy	6.75	1.53	Slight	7.00	1.48	Slight
4	15	7.20	1.65	Consid- erable	7.70	1.73	Slight	7.70	1.63	Consid- erable
4	16	6.60	1.58	Consid- erable	7.55	1.76	Slight	7.60	1.69	Consid- erable
4	17	5.75	1.66	Heavy	7.50	1.87	Slight	7.65	1.72	Consid- erable
4	18	5.10	1.77	Heavy	7.40	1.97	Slight	7.50	1.83	Consid- erable
5	19	5.05	1.80	Heavy	7.60	2.00	Consid- able	7.70	1.89	Consid- erable
5	20	4.90	1.81	Heavy	7.40	2.11	Heavy	7.70	1.92	Consid- erable

TABLE IV
Protein breakdown of casein by *P. roqueforti* (Culture 32) at different hydrogen-ion concentrations

EXPERIMENT	1		2		3	
	23.0° C. (Approx.)		24.0° C.		24.0° C.	
	Control	Inoculated	Control	Inoculated	Control	Inoculated
Standard Salt Media +	Casein 3% Dextrose 18% CaCO ₃ .8%		Casein 3% Dextrose 18% CaCO ₃ .8%		Casein 2% Dextrose 16% CaCO ₃ Nil	
Incubation temp.	23.0° C. (Approx.)		24.0° C.		22.5° C.	
Inoculation	Control	Inoculated	Control	Inoculated	Control	Inoculated
Days growth	Nil	9	Nil	14	Nil	11
pH of filtrate	6.20	6.35	6.05	7.25	6.25	3.00
	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.
	Nitrogen in 100 ml.	Nitrogen in 100 ml.	Nitrogen in 100 ml.	Nitrogen in 100 ml.	Nitrogen in 100 ml.	Nitrogen in 100 ml.
Soluble nitrogen (water soluble)	403.0	107.0	378.0	74.0	274	61.4
Protease nitrogen (sodium sulphate precipitate)		8.20	1.48	6.96	.53	2.81
Peptone nitrogen (tannic acid precipitate)		45.64	16.36	18.58	6.63	24.33
Sub. peptone nitrogen (tannic acid filtrate)		42.16	42.16	45.26	42.04	32.86
Filtrate 3A { Protease Sub. peptone	55.2	96.0	60.0	70.8	52.0	60.0
Soluble nitrogen (acetic acid filtrate)	75.0	100.5		75.5	68.0	60.3
"Amino" nitrogen phosphotungstic filtrate	15.0	43.5	24.0	36.0	15.9	35.25

3. The pH of the filtrate, shows that in all cases during the latter stages of growth the medium is returning from acid to neutral or alkaline reaction. This change is more marked in the flasks showing growth between the 14th and 17th day.

4. The presence or absence of dextrose in the medium after growth shows the same general trend as in the previous experiments. However, the considerable quantity of dextrose, which attaches to the sides of the Erlenmeyer flasks when preparing and sterilizing media of such high dextrose concentrations, makes a qualitative determination of uncertain value.

5. *P. roqueforti* is capable of growing well in media with high concentrations of casein and dextrose. The dry felts obtained from such growth amount to as much as 8 per cent of the media.

The Protein Breakdown of Casein by P. roqueforti at Different Hydrogen-ion Concentrations

From the previous experiments it is seen that the hydrogen-ion concentration during growth could be considerably varied. With the production of an enzyme extract in mind it was considered desirable to determine the difference in the protein breakdown during growth under variable conditions of pH. Two media were planned, namely, (1) standard salts; 3 per cent casein; 18 per cent dextrose, and 0.8 per cent CaCO_3 to maintain a reaction around neutral during growth. (2) Standard salts; 2 per cent casein; 16 per cent dextrose to produce and maintain a strongly acid reaction during growth.

Figure 1 shows the change of reaction of these two media during the growth of *P. roqueforti* and the final reaction on the day for analysis is given in table IV. Both show the desired reaction was obtained.

The Erlenmeyer flasks of 125 ml. were prepared with 25 ml. of medium in each as stated. The weight of each flask was recorded before sterilization so that loss by evaporation could be made with distilled water just before analysis. After sterilization the flasks were inoculated and incubated as stated in table IV. At the end of the growth period the flasks were made up to their original weight with water and filtered.

The filtrate was analyzed for the distribution of nitrogen and though the data are not extensive the following trends are shown in table IV:

1. By comparison with the soluble nitrogen in the control it is seen that four to six times as much nitrogen is present in the felt as in the filtrate.

2. Considering the higher percentage of casein in the media of Experiment 2, a higher percentage of the total soluble nitrogen is found in the acid filtrates.

3. The soluble nitrogen in Experiment 2 (nearly neutral reaction) increased with a longer period of growth, though the amount in each case is less than the control. The opposite is found to be the case where the mold is growing in an acid medium. (Experiments 3 and 4).

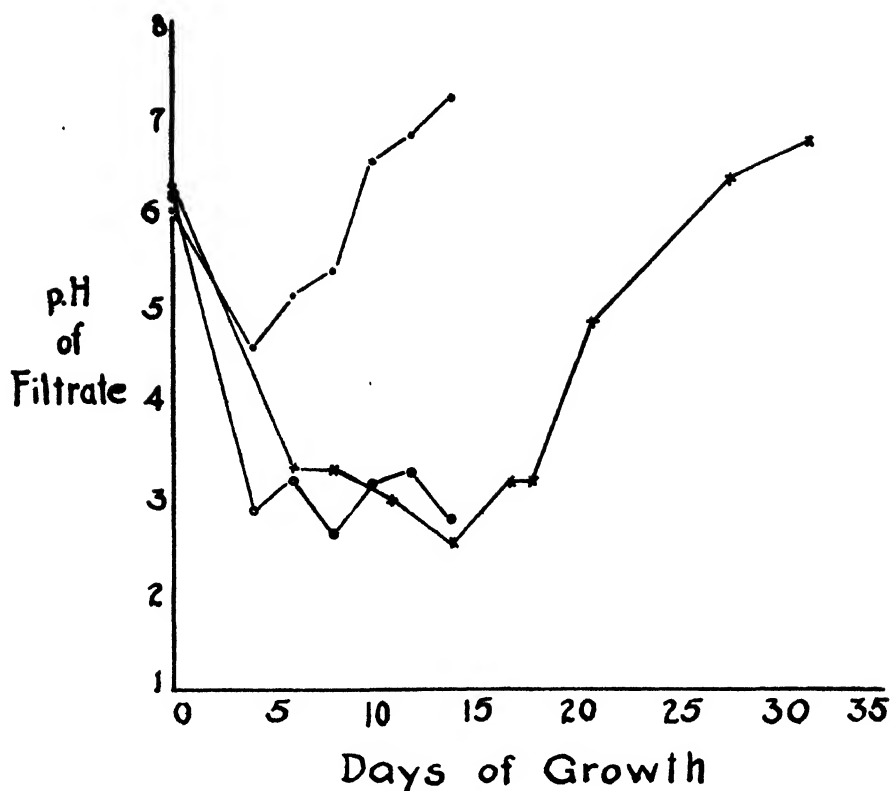


FIG. 1. pH of Filtrate at various ages of growth of *P. roqueforti* (culture 32).

- Experiment 2; Table IV; Casein 3%; Dextrose 18%; CaCO_3 .8%
- Experiment 3; “ IV; “ 2%; “ 16%; “ Nil.
- × Experiment 4; “ IV; “ 2%; “ 16%; “ Nil.

4. The proteose nitrogen is somewhat greater in the filtrate where growth has taken place. This figure is increased by a longer period of growth.

5. The amount of proteose nitrogen is not sufficient to conclude a marked difference between the very acid and almost neutral filtrate.

6. The peptone nitrogen shows a definite increase over the control due to the growth of *P. roqueforti*. This increase is reduced with the longer period of growth.

7. With the peptone nitrogen there is no significant difference in the results obtained between the very acid and the almost neutral filtrates.

8. The sub-peptone nitrogen shows a definite decrease from the control in the earlier growth at 10 and 11 days. In the case of Experiments 2 and 4 this was increased when the analysis was made at a later period of growth.

9. The sub-peptone nitrogen increased much more rapidly by the growth of *P. roqueforti* in the almost neutral medium than in the acid filtrate.

10. The amino nitrogen, phosphotungstic filtrate shows a tendency to increase over that of the control.

11. From the small amount of data given it would appear that the pH of the medium during growth did not materially change the type of the protein breakdown of casein by the growth of *P. roqueforti* for the growth period studied. However, there is an indication that the protein breakdown would be materially changed were the period for growth extended.

DISCUSSION OF RESULTS

The work reported in this paper shows that with suitable salts and organic nutrients of casein and dextrose a very abundant growth of *P. roqueforti* can be obtained. The maximum growth was dependent on having the casein and dextrose in the right proportion, the mold requiring about six times as much dextrose as casein. A medium of about 25 per cent solids gave the highest felt weight.

As has been previously shown by other workers with bacteria (6) (3) and molds (12) (1) the reaction of the medium was changed during the growth of *P. roqueforti*. This change of reaction may be reversible, depending on the composition of the medium. The growth of *P. roqueforti* on the casein medium free from dextrose causes the reaction to go to the alkaline side. The growth of *P. roqueforti* on the casein, dextrose media causes an acid reaction to develop till all the dextrose is used up, the reaction then reversing towards the alkaline side. The extreme range of pH in these experiments was between pH 2.2 to pH 7.7. However, the change of pH of the media was limited in some cases by the use of precipitated chalk or a suitable balance between dextrose and casein, or a combination of both.

In a study of the protein breakdown of casein under conditions of high and low pH, protein fractionation of the filtrate showed that the subpeptone nitrogen was formed much more rapidly by the growth of *P. roqueforti* in the almost neutral medium than in the acid medium. Other protein fractions did not show marked differences.

The enzymes elaborated by *P. roqueforti* in these experiments have not been studied but according to the investigations of Naylor *et al.* (15) there would be very considerable enzyme formation. However, the obtaining of an enzyme concentrate suitable for commercial application to the processed cheese industry will undoubtedly require very considerable investigation before its application can hope to be recommended. ✓

SUMMARY

With the object in view of obtaining a strong enzyme extract to produce a Roquefort flavor in processed cheese subsequent to heating, methods for obtaining a heavy growth of the mold on synthetic media have been investigated. A specially heavy growth of the mold *P. roqueforti* was obtained

by using a modified Dox (7) salt solution and increasing the proportion of casein and dextrose. Maximum felts were obtained at 5 per cent casein and 20 per cent dextrose.

The change of pH during growth is most extensive, ranging from pH 2.05 to 7.7 depending on the proportion of dextrose and casein. While the dextrose was present in the medium an acid reaction was obtained. The reaction moved towards the alkaline side as soon as the dextrose was used up by the mold.

A preliminary study of the protein breakdown of the casein in the media under widely different hydrogen ion concentrations was made. Thus far it would appear that the pH of the medium during growth did not materially change the type of the general protein breakdown of the casein by the growth of *P. roqueforti* for the growth periods studied.

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REFERENCES

- (1) ARMSTRONG, GEO. M. Studies in the physiology of the fungi, XIV. Sulphur nutrition: The use of thiosulphate as influenced by hydrogen ion concentration. *Ann. Mo. Bot. Gard.* VIII, 237-281. 1921.
- (2) AYERS, S. HENRY. Casein media adapted to milk analysis. *Science*, N. S., XXXV, 222-240. 1912.
- (3) AYERS, S. H., and RUPP, P. Simultaneous acid and alkaline bacterial fermentations from dextrose and the salts of organic acids respectively. *Jour. of Infect. Diseases*, XXIII, 188-216. 1918.
- (4) BENSON, J. The manufacture of Stilton and Wensleydale cheese. *Jour. British Dairy Farmers Assn.*, XXV, 13-20. 1911.
- (5) BUCHANAN, R. E., and FULMER, ELLIS I. Physiology and biochemistry of bacteria. Vol. III, Effect of microorganisms upon environment, fermentation and other changes produced. Williams & Wilkins Co. 1930.
- (6) CLARK, W. M., and LUBS, H. A. The differentiation of bacteria of the colonaerogenes family by the use of indicators. *Jour. of Infect. Diseases*, XVII, 160-173. 1915.
- (7) DOX, A. W. The intracellular enzymes of *Penicillium* and *Aspergillus*. *Bul.* 120, U. S. Dept. Agr. Bureau of Animal Ind. 1910.
- (8) DUGGER, B. M., SEVERY, J. W., and SCHMITZ, H. Studies in the physiology of the fungi. IV. The growth of certain fungi in plant decoctions. Preliminary account. *Ann. Mo. Bot. Gard.*, IV, 165-173. 1917.
- (9) EAGLES, B. A., and SADLER, W. 36. Cheese-ripening studies¹. Nitrogen distribution in Kingston cheese-ripening. *Jour. of Dairy Resch.*, III, 227-240. 1932.
- (10) GOLDING, N. S. Wensleydale cheese. Part I. Manufacture on the coast of British Columbia. In manuscript. 1924.

- (11) GOLDING, N. S. The mold associated with the ripening of blue-veined cheese. *Mycologia*, XVII, 19-32. 1925.
- (12) JOHNSON, HARLAN W. Relationships between hydrogen ion, hydroxyl ion and salt concentrations and the growth of seven soil molds. *Resch. Bul.* 76, Iowa St. Col., Ames. 1923.
- (13) KERTESZ, ZOLTAN I. Method for the estimation of enzyme yield in fungus cultures. *Jour. of Bio. Chem.*, XC, 15-23. 1931.
- (14) KNAPP, H. Studien uber die amglasebildung bei einigen *Aspergillus* arten. *Centralbt. Bakt. etc.* 2 Abt. (15/24), 372-398. 1927. *From Biological Abst.* IV, 7346. 1930.
- (15) NAYLOR, N. M., WEISBRODT, SMITH L., and COLLINS, HELEN JO. The esterase and protease of *Penicillium Roqueforti*. *Jour. of Sci. Iowa*, IV, 465-471. 1930.
- (16) ORLA-JENSEN, S. The lactic acid bacteria (in English). *D. Kgl. Danske Vidensk. Selsk. Skrifter, Naturv. og Mathematisk Afd.* 8 Rackke V. No. 2, Copenhagen. 1919.
- (17) ORLA-JENSEN, S. (trans. by Arup). *Dairy Bacteriology*, P. 132, J. and A. Churchill. 1921.
- (18) PERCIVAL, J., and MASON, G. HEATHER. The micro-flora of Stilton cheese. *Jour. Agr. Sci.* V, Part 2, 222-229. 1913.
- (19) PIRTLE, T. R. History of the dairy industry. *Mojonnier Bros. Co.* 1926.
- (20) SMYTH, H. F., and OBOLD, W. L. *Industrial Microbiology*. The Williams and Wilkins Co. 1930.
- (21) THOM, CHARLES, and CHURCH, MARGARET B. *The Penicillia*. Williams and Wilkins Co. 1930.
- (22) THOM, CHARLES, and CURRIE, JAMES N. The dominance of Roquefort mold in cheese. *Jour. Bio. Chem.*, XV, 249-258. 1913.
- (23) WASTENEYS, H., and BORSOOK, H. A method for the fractional analysis of incomplete protein hydrolysates. *Jour. Biol. Chem.*, LXII, 1-14. 1924.

A COMPARISON OF THE LEUCOCYTE COUNT, THE BROM THYMOL BLUE REACTION AND THE CATALASE CONTENT OF FRESHLY DRAWN MILK

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INTRODUCTION

The distinguishing characteristics between normal and abnormal freshly drawn milk constitutes a study that has received a renewed interest during the past few years. The results of recent investigations have thrown considerable doubt upon certain of the earlier studies in which milk samples classed as normal and drawn from apparently normal udders were used. In the light of our present knowledge it appears very probable that a considerable percentage of the milk samples from such udders would have given positive reactions to certain chemical tests recognized today as indicating abnormal udder conditions.

Udall and Johnson (14), Hucker, Trudell and Jennings (7), Hucker and Udall (8), and Hucker (9) have emphasized the necessity of ascertaining the physical condition of the udder from which the milk is drawn before classifying it as coming from a normal udder or as being a normal product. These investigators have shown that "apparently normal udders" may show signs of infections in the form of indurations or fibrotic tissue and that the milk coming from such quarters may be abnormal in reaction, in the amount of catalase present and in the chloride and lactose content.

The prevailing conception of the numbers of leucocytes present in normal milk drawn from apparently healthy udders is based upon studies in which the samples were considered normal by their physical properties only. Chemical determinations were not considered necessary in order to establish the normal or abnormal status of these milks.

The object of the present study was to secure information on the leucocyte content of milk in relation to the pH value, the catalase content and to the presence or absence in the producing animal of pronounced positive evidence of mastitis during a period of several months duration.

HISTORICAL

Among the early reports on the leucocyte content of normal milk are those of Cooledge (4), Breed (2), Tweed (13) and Copeland and Olson (5). The average numbers per cc. reported by these investigators were 930,000; 868,000; 626,000; and 657,000 respectively.

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Cherrington, Hansen and Halversen (3) reported the leucocyte count of milk from six normal cows to be 43,000 per cc. as compared to 3,000,000 per cc. for the milk from seven cows known to be suffering from mastitis. They concluded that milk from normal udders usually contains less than 50,000 cells per cc. whereas milk from infected udders almost invariably contains more than 100,000 per cc. Hucker, Trudell and Jennings (7) made an extensive study of the various methods for the diagnosis of mastitis. They reported that no significance could be attached to the leucocyte content when it was less than 3,000,000 per cc. In a more recent study Hucker and Udall (8) found that a cell count of more than 500,000 per cc. always indicated an abnormal or pathological condition of the udder. Still more recently Hucker (9) states that all quarters showing cell counts of more than 150,000 per cc. should be considered as suspicious, if not definitely proved to be infected. He found no quarter free from indurations or fibrotic tissue to discharge more than 150,000 leucocytes per cc. Ninety-nine per cent of the quarters free from scar tissue produced milk having less than 60,000 per cc.

Wayne and Macy (15) recently reported an average cell count of 1,252,000 per cc. for 284 samples of normal milk. They made no statement relative to their methods of determining the normal status of their samples.

During the past few years numerous studies have been made of the hydrogen-ion concentration of milk, especially as it relates to the diagnosis of mastitis and to the detection of milk from infected udders. Baker and Van Slyke (1) developed a colorimetric method for the detection of abnormal milk in which brom cresol purple was used as the indicator to determine the hydrogen-ion concentration. In recent years brom thymol blue has replaced brom cresol purple. This determination is known as the brom thymol blue test, commonly called the thybromol test.

Rosell (12) and Udall and Johnson (14) have made extensive studies on the value of the brom thymol blue test as a means of diagnosing mastitis. Rosell (12) considers it to be one of the most useful and easily applied methods. He found it to be approximately 90 per cent accurate. Udall and Johnson (14) state that the presence of a definite color change always means mastitis when applied to the milk of cows that are not in the advanced stages of lactation or that have not just freshened. They consider it a highly reliable test for the diagnosis of chronic mastitis. Hucker, Trudell and Jennings (7) found that positive reactions to the brom thymol blue test always indicated an infected quarter, but negative results did not assure a normal udder. However, they considered an endpoint of pH 7.0 to represent the dividing line between a normal and an abnormal reaction.

Milk from an infected quarter usually has a higher content of the enzyme, catalase, than does milk from a healthy udder. Gratz and Naray (6) were among the early investigators to report on the value of the catalase test in detecting mastitis. They found the test to be accurate if combined with the leucocyte count. Such was necessary in order to exclude the presence of blood as the cause of the increased catalase content. Hucker, Trudell and Jennings (7) found the catalase test to be an exceedingly delicate method of selecting cows that carry an active udder infection. Hucker (9) reported the catalase test to be highly efficient in detecting both clinical and sub-clinical mastitis. The increase over that of normal usually varies with the extent of the infection.

METHODS

The data presented in this paper represent a comparative study of the brom thymol blue test, the catalase test and the leucocyte count of 1019 samples of freshly drawn milk from individual udder quarters of 40 cows. Samples were collected from most of the animals at intervals varying from one to three weeks and extending over a period of four to five months. Milk from cows in the advanced stages of lactation, during the last three months of the milking period, or that had just freshened was excluded from this study. Such milk usually has a decreased hydrogen-ion concentration and an increased catalase and leucocyte content. These changes are due to physiological rather than to pathological conditions.

The milk samples used in this study were samples of the fore milk and were drawn after the initial four or five streams had been discarded. They were brought to the laboratory immediately for examination.

Leucocyte counts were made by the direct microscopic method as developed by Prescott and Breed (11). Newman's (10) formula No. 2 was used in staining the preparations. Twenty fields of each smear were counted.

The brom thymol blue test was made by adding 1.0 cc. of a 0.04 per cent aqueous solution of the indicator to 5.0 cc. of milk. Normal fresh milk gives a yellowish-green color and has a pH value 6.3 to 6.8. Milk drawn from infected udders usually turns light green, green or dark green, depending on the activity of the inflammatory process. Such milk has a pH value greater than 6.8.

The catalase test was made by mixing 15 cc. of fresh milk and 5.0 cc. of a 1.0 per cent solution of hydrogen peroxide in a fermentation tube. The mixture was incubated at 37 degrees C. for two hours, after which time the amount of liberated oxygen was recorded. The liberation by the catalase of more than 1.5 cc. of oxygen from 5.0 cc. of a 1.0 per cent solution of hydrogen peroxide in 15.0 cc. of freshly drawn milk is generally regarded as indicating an abnormal catalase content.

PRESENTATION OF DATA

The grouping of the samples, based on the leucocyte content, the brom thymol blue and the catalase tests are shown in table 1.

TABLE 1

Leucocyte counts, brom thymol blue reactions and catalase determinations of freshly drawn milk

LEUCOCYTES PER C C.	NO OF SAMPLES	BROM THYMOL BLUE TEST NORMAL		CATALASE TEST CC. OF LIBERATED OXYGEN					NORMAL TO BOTH BROM THYMOL BLUE AND CATALASE TEST
		No	%	0-1.5*		1 5-3 0	3 0-5 0	5 0 +	Per cent
				No.	%				
Less than 100,000	513	508	99.0	450	88.5	45	17	1	87.7
100,000- 250,000	214	203	94.9	143	66.5	50	18	3	65.5
250,000- 500,000	91	72	79.0	36	39.5	28	13	14	37.4
500,000- 750,000	58	28	48.4	18	31.0	14	10	16	24.1
750,000- 1,000,000	50	22	43.0	4	8.0	22	10	14	8.0
1,000,000- 2,000,000	29	5	17.3	1	3.9	2	9	17	.0
Over 2,000,000	64	12	18.7	2	3.1	6	8	48	2.1
Total	1019	850	83.4	654	64.3	167	85	113	63.3

* Normal catalase index.

DISCUSSION OF RESULTS

From a study of table 1, it is seen that a general relation exists between the leucocyte content, and the reaction of the milk samples to the brom thymol blue and catalase tests. As the cellular content increases, there is a corresponding decrease in the percentage of samples giving normal reactions to both these tests. The decrease from normal is more pronounced when measured by the catalase test than when measure by the brom thymol blue test.

Of the samples having leucocyte counts of 250,000-500,000; 500,000-750,000; 750,000-1,000,000; and 1,000,000-2,000,000 per cc., only 79.0 per cent, 48.4 per cent, and 43.0 per cent and 17.3 per cent, respectively, were found to be normal to the brom thymol blue test.

The results secured by the catalase determinations are of interest. Eighty-eight and five-tenths per cent of the samples having leucocyte counts of less than 100,000 per cc. were found to have a catalase index of less than 1.5 cc. of liberated oxygen as compared to 3.9 per cent for the samples ranging in leucocyte count from 1,000,000 to 2,000,000 per cc. In 59 per cent of the samples of the latter group, the catalase index was in excess of 5.0 cc. of liberated oxygen. Samples having leucocyte counts in excess of 2,000,000 per cc. responded to both the catalase and brom thymol blue tests in a manner similar to the samples ranging from 1,000,000–2,000,000 leucocytes per cc.

Eighty-three and four-tenths per cent of the samples gave normal reactions to the brom thymol blue test as compared to 64.3 per cent for the catalase test. Of the 850 samples reacting normal to the brom thymol blue test, only 77 per cent had a normal catalase index. Occasionally a sample was found to give an abnormal reaction to the brom thymol blue test and yet have a catalase index within normal limits. The per cent of samples in each group reacting normal to both tests is shown in the last column of table 1. For the corresponding group of samples, these figures are slightly lower than those for the catalase reaction alone.

The average leucocyte counts of the 850 samples showing negative reactions to the brom thymol blue test and for the 654 samples reacting negatively to the catalase test were 208,000 and 151,000 per cc. respectively. At one time or oftener during the period over which this study extended nine of the animals showed pronounced positive evidence of mastitis as indicated by the production of stringy, or flaky milk that soon separated on standing. Such milk was usually nearly neutral or slightly alkaline in reaction and contained a high cellular content. The average leucocyte content of all samples of milk coming from these nine animals was found to be 2,800,000 per cc. When all samples of milk drawn from such animals were excluded, an average leucocyte content of 225,000 was obtained for 870 samples collected from 122 udder quarters of 31 cows. When all samples of milk from udder quarters, that at any time during the observation period gave milk reacting abnormally to the brom thymol blue test were excluded, an average leucocyte count of 160,000 per cc. was found for 700 samples from 103 udder quarters of 29 cows.

Nineteen cows produced milk throughout the observation period in one or more quarters, that at all times contained a normal catalase content. There were 247 samples in this group coming from 39 udder quarters. The average leucocyte count for these samples was found to be 72,000 per cc. Only four of the nineteen cows produced milk of such low catalase content from all quarters.

SUMMARY AND CONCLUSIONS

A comparative study of the brom thymol blue reaction, the catalase test and the leucocyte content was made of 1019 samples of freshly drawn milk. Milk samples from cows in the advanced stages of lactation or that had just freshened were not included in this study.

Based on the leucocyte content per cc. the samples were grouped as follows: less than 100,000; 100,000-250,000; 250,000-500,000; 500,000-750,000; 750,000-1,000,000; 1,000,000-2,000,000; and over 2,000,000.

In the order of the above increasing leucocyte content, the following percentage of the various groups of samples reacted normally to the brom thymol blue test: 99.0, 94.9, 79.0, 48.4, 43.0, 17.3 and 18.7. In each group a smaller percentage of the samples reacted normally to the catalase test than to the brom thymol blue test. These respective percentages were as follows: 88.5, 66.5, 39.5, 31.0, 8.0, 3.9 and 3.1.

An average leucocyte count of 225,000 was obtained for 870 samples of milk drawn from 31 cows that at all times during the period of observation were free from pronounced positive evidence of mastitis as indicated by the production of stringy or flaky milk. The average cell count of 850 samples reacting negatively to the brom thymol test was 208,000 per cc. as compared to 151,000 for the 654 samples having a catalase index of less than 1.5 cc. The average leucocyte count of 700 samples coming from 103 udder quarters that at all times during the period of observation produced milk giving a normal reaction to the brom thymol blue test was found to be 160,000 per cc. Thirty-nine udder quarters from 19 cows produced milk that at no time showed an excessive catalase content. The average cell count for 247 samples of such milk was found to be 72,000 per cc.

The results of this study indicate that the percentage of animals producing milk, that is normal to both the brom thymol blue and catalase tests, from all quarters and at all times is low.

The average cell count of milk drawn from healthy normal udders is very much less than the average cell count of normal milk as reported by the earlier investigators and by some of the more recent contributors to the subject.

REFERENCES

- (1) BAKER, J. C. AND VAN SLYKE, L. L. A method for the preliminary detection of abnormal milk. New York (Geneva) Agr. Exp. Sta. Tech. Bul. 71. 1919.
- (2) BREED, R. S. The sanitary significance of leucocytes in milk. New York (Geneva) Agr. Exp. Sta. Bul. 568. 1929.
- (3) CHERINGTON, V. A., HANSEN, H. C. AND HALVERSEN, W. V. The leucocyte content of milk as correlated with bacterial count and hydrogen-ion concentration for the detection of mastitis. JOURNAL OF DAIRY SCIENCE 16: p. 59-69. 1933.
- (4) COOLEGE, L. H. Studies upon the bacterial flora of samples of milk with cellular counts as drawn from apparently normal udders. Mich. Agr. Exp. Sta. Tech. Bul. 41. 1918.

- (5) COPELAND, LYNN AND OLSON, T. M. The bacterial flora of normal cows' udders. South Dakota Agr. Exp. Sta. Bul. 218. 1926.
- (6) GRATZ, O. AND NARAY, A. Comparative investigations in regard to the utility of the catalase, reductase and leucocyte tests for detecting mastitis milk. Milchw. Zentbl. V. 41, p. 225-232, 257-263, 289-303. 1912. Original not seen; reviewed in Exp. Sta. Record, V. 28, p. 680.
- (7) HUCKER, G. J., TRUDELL, FERDINAND AND JENNINGS, WILLIAM S. Mastitis: I, The incidence and detection of sub-clinical streptococcus mastitis. New York (Geneva) Agr. Exp. Sta. Bul. 199. 1932.
- (8) HUCKER, G. J., AND UDALL, D. H. Relation between the presence of fibrotic tissue in the udder and streptococci or cells in freshly drawn milk. The Cornell Veterinarian, 23: p. 32-39. 1933.
- (9) HUCKER, G. J. Relationships of leucocytes and streptococci to fibrosis of the udder. Amer. Jour. of Public Health, 23: p. 237-245. 1933.
- (10) NEWMAN, R. W. A one solution of technique for the direct microscopic method of counting bacteria in milk. Monthly Bul. 16, California State Dept. of Agr., p. 1-9. 1927.
- (11) PRESCOTT, S. C., AND BREED, R. S. The determination of the number of body cells in milk by a direct method. Jour. Inf. Diseases, 7, p. 632-40. 1931.
- (12) ROSELL, J. M. Some results of three years of mastitis studies in the Province of Quebec. 20th Ann. Report of the International Assn. of Dairy and Milk Inspectors, p. 292-308. 1931.
- (13) TWEED, R. L. The relation of high cellular counts to bacterium abortus infections of the udder. Mich. Agr. Exp. Sta. Tech. Bul. 61. 1923.
- (14) UDALL, R. H., AND JOHNSON, S. D. The diagnosis and control of mastitis. The Cornell Veterinarian, 21: p. 190-206. 1931.
- (15) WAYNE, RALPH AND MACY, H. The effect of various methods for drying up cows on the bacterial and cell content of milk. JOURNAL OF DAIRY SCIENCE 16: p. 79-91. 1933.

THE EFFECT OF DAILY MASSIVE DOSES OF VIOSTEROL UPON CALCIUM AND PHOSPHORUS METABOLISM AND BLOOD CALCIUM AND INORGANIC PHOSPHORUS IN CALVES¹

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The beneficial effect of the administration of normal doses of cod-liver oil or irradiated ergosterol in young calves has been definitely established but the effect of abnormally large doses of irradiated ergosterol in young calves is relatively unknown.

This study was undertaken to observe the possible toxic symptoms and pathologic results produced by excessive daily doses of viosterol in calves on a normal and on a high calcium and phosphorus intake. Calcium and phosphorus balances were determined upon two young healthy calves on a natural ration of whole milk. The blood calcium and inorganic phosphorus values of these same animals were studied together with values obtained from two other young calves on a whole milk ration but on a lower calcium, phosphorus and viosterol intake.

Several observations prior to and since our experiments have indicated that the degree of toxicity of irradiated ergosterol is partially dependent upon the calcium-phosphorus ratio of the diet (1) (2) (3) (4). Young calves are normally on a diet of whole milk in which this ratio is approximately 1:1. In order to supply the metabolism animals with an abundance of calcium and phosphorus, the ration was supplemented with enough mono-calcium phosphate to bring the total intake of these elements to 15 grams of each per day.

The results of metabolism studies have been reported on rats (5) (6) (7) (8), rabbits (9), dogs and infants (10), therefore an extensive review of this literature is not necessary.

EXPERIMENTAL

Effect of 1000X Viosterol—Preliminary to the metabolism experiment, two young calves (V1 and V2, about 14 days of age) were used to determine the pyramiding effect of heavy viosterol feeding when on a normal diet. Each animal received approximately 10 pounds of milk per day which contained 5.0 grams of calcium and 3.9 grams of phosphorus. In

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addition to the milk they received 25 cc. of Mead Johnson and Company viosterol which had an antirachitic potency of 1000X according to their standardization.² The calves were on experiment for 27 days, at which time V2 died as a result of the administration of the viosterol and V1 was slaughtered. Definite overdosage symptoms were not particularly noticeable during the first seven days but after that time the calves became drowsy and were apathetic. It became increasingly difficult to get them to drink the entire 10 pounds of milk and toward the end of the experiment they would not drink more than 6 pounds each per day. At times the feces were very soft and contained some blood but severe diarrhea was not observed.

Blood samples were drawn daily from the jugular vein for 17 days, after which the intervals were lengthened. The blood was analyzed for calcium and inorganic phosphorus by methods used in previous work (11).

At autopsy V2 showed marked hemorrhagic gastritis, V1 displayed the same symptoms but to a lesser degree. The kidneys of both calves were soft and enlarged. The bile sac was removed from each calf, the contents weighed and calcium, phosphorus and magnesium were determined on each sample. The ash content of the right dental pad was determined on V1 and V2 and was found to be 54.1 and 57.7 per cent respectively.

Effect of 2500X Viosterol—The animals used in the metabolism investigation were healthy, young male calves, V3 and V4, approximately 30 days of age. They were placed in individual metabolism stalls and fed the basal ration for five days prior to the oral administration of viosterol. The basal ration consisted of 14 pounds of whole milk, supplemented with enough $\text{CaH}_4(\text{PO}_4)_2 \cdot \text{H}_2\text{O}$ to bring the total calcium intake to 15 grams per day. A suspension of 25 grams of the salt was made with about 3 pounds of milk and was given to the calves morning and night. This was followed by feeding 4 pounds of milk. The ration was calculated to supply an excess of calcium and phosphorus to the animals and also, to keep the ratio of these elements about 1:1. After a period of five days, 50 cc. of 2500X viosterol was fed to each calf daily.

Feces and urine were collected separately in 24 hour periods. Each daily sample was weighed or measured, thoroughly mixed and sampled for analysis. The excreta were analyzed each day for calcium and phosphorus as in previous work (12). A composite sample of the milk contained 0.1101 per cent calcium and 0.0855 per cent phosphorus.

DISCUSSION OF RESULTS

Blood Calcium and Inorganic Phosphorus

The effects of the two levels of viosterol feeding on the blood pictures of the four calves are shown in table 1. It is of interest to note that these

² We are indebted to Mead Johnson and Company for the viosterol used in these experiments.

TABLE 1

The calcium and phosphorus content of the blood, mgm. per 100 cc. of plasma

DATE	V1		V2		DATE	V3		V4	
	Ca	P	Ca	P		Ca	P	Ca	P
May 28*	11.5	6.51	12.1	6.68	Oct. 25*	12.7	6.84	12.2	6.86
29	11.9	6.38	11.9	7.10	26	13.2	5.87	11.9	6.62
30	13.6	6.19	12.6	6.48	27	12.8	6.62	12.5	7.71
31	13.6	7.40	12.3	7.23	28	14.2	7.35	13.1	8.87
June 1	13.0	7.27	13.0	7.44	29	12.8	7.86	12.2	9.13
2	12.3	7.53	12.5	8.12	30	14.4	8.01	12.8	7.96
3	13.1	7.14	12.8	8.17	31	13.0	12.40	12.6	10.20
4	11.8	7.62	12.6	7.35	Nov. 1	Died		14.3	10.95
5	12.2	7.76	12.3	6.95	2			13.9	10.45
6	13.4	8.12	12.2	7.14	3			13.0	9.80
7	12.7	8.01	11.6	5.66	4			12.8	11.10
8	13.0	8.87	11.8	5.19	5			13.1	10.70
9	13.6	8.87	11.4	4.96	6			13.9	9.22
10	12.7	8.23	11.8	5.10	7			12.8	8.90
11	13.6	8.81	12.1	5.81	8			13.8	8.87
12	12.7	7.91	11.9	5.21	9			14.1	9.84
13	13.2	8.01	13.2	5.97	10			10.7	11.54
16	12.2	8.33	11.7	6.65				Killed	
19	10.5	7.62	11.0	7.02					
24	10.8	8.33	10.0	8.01					
	Killed		Died						

* Average of three separate samples prior to feeding viosterol.

young calves did not show marked hypercalcemia when fed large doses of viosterol. However, the blood calcium did increase in a moderate way and had a tendency to reach a maximum value within 72 hours from the time the viosterol was first fed. The pyramiding effect was entirely absent.

The inorganic phosphorus values had a tendency to decrease slightly during the first 24 hours immediately following the first large dose of viosterol. After the initial drop the phosphorus values increased to a maximum but at a slower rate. If the animals remained on experiment for 14 days or longer the blood calcium and phosphorus had a tendency to return to their pre-viosterol feeding values.

The decided terminal drop in the blood calcium curves and the decided increase in the blood phosphorus values of V2 and V3 some hours before death are of interest. V4 displayed the same general blood phenomenon at the time it was destroyed. Collip (13) and Macleod and Taylor (14) have called attention to the terminal decline in the calcium curve caused by parathyroid overdosage in dogs. One of us (15) has previously indicated that the blood calcium curves of calves correspond to this same typical form when fatal overdosage symptoms are produced.

TABLE 2—(Continued)

DATE	WEIGHT	VIOS- TEROL 2500X	CALCIUM			PHOSPHORUS				Ca/P			
			Outgo		Intake	Balance	Used	Outgo			Intake	Balance	Used
			Feces	Urine				Feces	Urine				
26	126	50	6.05	0.08	14.93	6.13	0.08	8.80	59.0	17.73	7.73	43.6	0.84
27	126	50	5.15	0.07	14.93	5.22	0.07	9.71	65.0	17.73	7.14	40.3	0.84
28	125	50	3.13	0.12	14.93	3.25	0.12	11.68	73.2	17.73	8.11	45.8	0.84
29	129	50	2.85	0.09	14.93	2.94	0.09	11.99	80.3	17.73	9.26	52.2	0.84
30	126	50	2.80	0.10	14.93	2.90	0.10	12.03	80.6	17.73	9.60	54.1	0.84
31	131	50	4.51	0.11	14.93	4.62	0.11	10.31	69.1	17.73	7.59	42.8	0.84
Nov.													
1	131	50	6.57	0.11	14.93	6.68	0.11	8.25	55.2	17.73	5.97	33.7	0.84
2	134	50	2.34	0.14	14.93	2.48	0.14	12.45	83.4	17.73	7.57	42.7	0.84
3	131	50	2.07	0.20	14.93	2.27	0.20	12.66	84.8	17.73	10.33	58.3	0.84
4	132	50	1.25	0.14	14.93	1.39	0.14	13.54	90.6	17.73	9.30	52.5	0.84
5	136	50	2.69	0.26	14.93	2.95	0.26	11.98	80.2	17.73	9.56	53.9	0.84
Average			3.58	0.13	14.93	3.71	0.13	11.22	75.1	17.73	8.38	47.3	0.84

Calcium and Phosphorus Balances

The results of the metabolism study are summarized in table 2. The balances for both animals are in fairly close agreement. Animal V3 developed severe diarrhea soon after the addition of viosterol to the ration which continued until the fatal termination. During the last three days the feces had a putrid odor, contained fat and occasionally small amounts of blood. V3 also manifested a lack of appetite soon after the viosterol was fed. In spite of this condition the animal made good use of both calcium and phosphorus. At autopsy V3 showed marked hemorrhagic gastritis, the true stomach contained a bloody curd and the rumen was filled with coagulated milk, $\text{CaH}_4(\text{PO}_4)_2 \cdot \text{H}_2\text{O}$ and viosterol. The kidneys were soft, enlarged and covered with large reddish gray spots. No abnormal calcification was visible by macroscopic examination. The bile sac in V3 was entirely empty.

Animal V4 reacted somewhat differently to the heavy feeding of viosterol. The appetite remained fairly good, the animal looked and acted normal and gained in weight. The viosterol feeding was continued for sixteen days, after which time the animal was killed and a post-mortem examination was made. During the last few days of the metabolism the feces became very soft or liquid, contained some fat and occasionally small blood clots. At autopsy, V4 showed the same general condition as V3 but, in addition, the lymph nodes along the backbone were usually large. The bile sac in V4 contained a small amount of bile, the analysis of which is given in table 4.

In general, the results of this metabolism show that the feeding of excessive doses of viosterol caused a decrease in the excretion of fecal calcium and phosphorus and markedly increased the excretion of urinary calcium under the conditions of this experiment. The animals remained in a high positive calcium and phosphorus balance because of the adequate food intake. The absorption of calcium and phosphorus from the intestinal tract was greater during the viosterol feeding period than during the fore-period. It is unfortunate that balances were not determined for nitrogen. Toward the end of the experiment, large albuminous precipitates formed in the urine indicating extensive renal damage. This observation is in agreement with Spies and Glover (16).

Some of the soft tissues were analyzed for calcium, phosphorus and magnesium to determine the relative amounts of these elements present. These results are presented in table 3.

The analyses of the various tissues of young normal calves are not available. However, Hess, Benjamin and Gross (17) and others have shown that calcium and phosphorus are present in these tissues in dogs in larger amounts than normal.

TABLE 3
Calcium, phosphorus and magnesium content of organs of calves fed large amounts of viosterol

	CALF V3			CALF V4		
	Ca	P	Mg	Ca	P	Mg
	Per 100 gm. dry tissue			Per 100 gm. dry tissue		
	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>
Liver	50.8	1023.0	82.0	37.0	1160.4	91.6
Kidney	250.0	1200.5	86.2	140.0	1169.0	97.4
Heart	128.3	857.8	87.2	27.1	477.3	57.5
Lymph-nodes	.			53.3	928.5	66.1
Aorta				301.7	390.0	19.8

The ash content of the right dental pad of V4 was found to be 55.8 per cent. The percentages of bone ash of these three calves are in agreement with values we have for normal animals. The ash content of the dental pads of rachitic calves show a much lower value (18). It is unlikely that any unusual amount of calcium or phosphorus was removed from the bones of the calves due to high viosterol feeding, because of the short time they were on experiment.

TABLE 4
Total milligrams of calcium, phosphorus and magnesium in the biles

	BILE	Ca	P	Mg
	<i>gm.</i>	<i>mg.</i>	<i>mg.</i>	<i>gm.</i>
V1	31.0	15.85	34.56	3.63
V2	46.0	12.19	41.16	3.59
V3	none			
V4	7.64	1.56	5.95	0.59

The grams of bile and the milligrams of calcium, phosphorus and magnesium in the bile of the three calves are given in table 4. V1 and V2 had about the normal quantity of bile for a young calf but the color and viscosity were abnormal. V3 and V4, which received the higher dosages of viosterol, had either no bile in the sac or only a small quantity of abnormal bile. The bile in all of the animals was characterized by a yellowish-brown color and high viscosity. These observations are of interest in view of the fact that the bile sacs of rachitic calves are unusually large and contain from 100 to 800 cc. of yellow to brick-red colored bile.

SUMMARY

The feeding of large daily doses of viosterol to young calves decreased the total excretions of calcium and phosphorus.

There was an increase in the absorption of calcium and phosphorus from the intestine. The excretion of calcium in the feces was decreased, whereas the excretion in the urine was greatly increased.

The concentration of calcium in the blood plasma was not as markedly increased as the inorganic phosphorus due to viosterol feeding. There was a tendency for the blood calcium to decrease and the phosphorus to increase some hours prior to death.

Calves apparently show an idiosyncrasy to the development of hypercalcemia under the conditions of this experiment.

REFERENCES

- (1) BILLS, C. E., and WIRICK, A. M. Long time feeding experiments with activated ergosterol. *Jour. Biol. Chem.* 86: 117. 1930.
- (2) SHOHL, A. T., GOLDBLATT, H., and BROWN, H. B. The pathological effects upon rats of excess irradiated ergosterol. *Jour. Clin. Investigation* 8: 505. 1930.
- (3) HARRIS, L. J., and INNES, J. R. M. The mode of action of vitamin D.—Studies on hypervitaminosis D. The influence of the calcium-phosphate intake. *Biochem. Jour.* 25: 367. 1931.
- (4) JONES, J. H., RAPOPORT, M., and HODES, H. L. The source of excess calcium in hypercalcemia induced by irradiated ergosterol. *Jour. Biol. Chem.* 89: 647. 1930.
- (5) WATCHORN, E. The absorption and excretion of calcium and phosphorus by rats receiving excessive doses of irradiated ergosterol. *Biochem. Jour.* 24: 631. 1930.
- (6) KERN, R., MONTGOMERY, M. F., and STILL, E. U. The effect of large doses of irradiated ergosterol upon nitrogen, calcium, and phosphorus metabolism in rats. *Jour. Biol. Chem.* 93: 365. 1931.
- (7) LIGHT, R. F., MILLER, G., and FREY, C. N. Studies on the effects of overdosage of vitamin D. *Jour. Biol. Chem.* 84: 487. 1929.
- (8) BROWN, H. B., and SHOHL, A. T. Rickets in rats. XI. The alteration of calcium and phosphorus metabolism of normal and rachitic rats produced by irradiated ergosterol. *Jour. Biol. Chem.* 86: 245. 1930.
- (9) ASHFORD, C. A. The phosphorus distribution in blood and the calcium and phosphorus excretion during hypervitaminosis D. *Biochem. Jour.* 24: 661. 1930.
- (10) HOTTINGER, A. Untersuchungen über bestrahltes Ergosterin. V. Stoffwechselversuche zur Ermittlung der biologischen Wirkungsweise. *Z. Kinderheilk.* 47: 341. 1929.
- (11) ROBINSON, C. S., and HUFFMAN, C. F. Studies on the chemical composition of beef blood. I. The concentrations of certain constituents in normal beef plasma. *Jour. Biol. Chem.* 67: 245. 1926.
- (12) ROBINSON, C. S., HUFFMAN, C. F., and MASON, M. F. The results of the ingestion of certain calcium salts and of lactose. *Jour. Biol. Chem.* 84: 257. 1929.
- (13) COLLIP, J. B. The parathyroid glands. *Medicine* 5: 1. 1926.

- (14) MACLEOD, J. J. R., and TAYLOR, N. B. Observations upon the effects produced in normal and parathyroidectomized dogs and herbivorous animals by injections of parathyroid extract. *Trans. Roy. Soc. Can.* 19: 27. 1925.
- (15) ROBINSON, C. S., HUFFMAN, C. F., and BURT, K. L. The effect of the administration of parathyroid extract on normal calves. *Jour. Biol. Chem.* 73: 477. 1927.
- (16) SPIES, T. D., and GLOVER, E. C. Renal lesions with retention of nitrogen products produced by massive doses of irradiated ergosterol. *Am. Jour. Path.* 6: 485. 1930.
- (17) HESS, A. F., BENJAMIN, H. R., and GROSS, J. The source of excess calcium in hypercalcemia induced by irradiated ergosterol. *Jour. Biol. Chem.* 94: 1. 1931.
- (18) DUNCAN, C. W., and HUFFMAN, C. F. Unpublished data. *Mich. Agr. Exp. Sta.*

PEDIGREE ANALYSIS AS A BASIS OF SELECTING BULL CALVES

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Proved sires have been advocated much during recent years as the soundest means of herd improvement. In view of the evidence advanced by critical research and the results of practical dairy experience, there seems to be little argument about this point. Yet, judging from progress now being made, it will be many years before any appreciable percentage of dairy calves are sired by good proved bulls.

For instance, the New York State Dairy Herd Improvement Association report for 1932 shows a total of 34,281 cows tested, yet only 134 bulls proved. Of this number only 25 were alive when proved. In any event, it appears that a great number of untried young bulls must continue to be used. Every proved sire was once an untried calf and some breeder had to take a chance in proving him. It is a tragedy and a severe loss to the industry that so many of these prospective herd improvers are failures when proved. Even when a young bull does prove out and effects an improvement in the herd, he is too often followed by another young sire that immediately reduces production back to its original low level and the actual improvement to the breed is negligible.

It is a serious challenge to constructive breeders and to students of breeding to uncover additional facts and supply information to dairymen that will materially assist them in the selection of untried bull calves, calves that will in the majority of cases prove out successful. Obviously, in the use of any untried young bull an element of chance is involved. The purpose of this study was to ascertain just what available information is needed by a breeder to lessen this element of chance and to be able to select a bull calf with a fair degree of certainty that he will at least maintain and if possible improve production in the herd.

The type of the calf and a study of his ancestry furnish the only basis for such selection. We know that the individual's conformation alone is not a reliable guide. Too many grand champion winners have developed into failures as sires of production for a great deal of dependence to be placed on this method. Consequently, the ancestry of the calf as shown by the pedigree remains the important basis for study.

In June of 1932 the American Jersey Cattle Club adopted a new classification for bulls, called "Tested Sires." To qualify as a "Tested Sire" it is required that a Jersey bull must have at least ten officially tested daughters with lactations of 270 days or more in length. The new classifica-

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tion displaced the old system of Register of Merit Sires which had been in operation for a number of years. This action was taken by the American Jersey Cattle Club as a result of extensive studies made by Gifford (1) and Gowen (2) and confirmed by research in the Club office (3). These studies indicated that the average production of a bull's first ten tested daughters furnishes a very good estimate of what his future progeny will produce. Edwards (4) in recently studying this problem by means of a different method of attack arrives at substantially the same conclusions.

The first volume of these "Tested Sires" was published in January 1933. This volume contains the names of 729 bulls and gives for each sire the total number of tested daughters and the average yield of all tested daughters computed to maturity. The present study is based on the data published in this book.

The plan of the investigation was to tabulate and examine carefully the performance of the ancestry of all the bulls listed in the "Tested Sire" volume and from such a study ascertain the possible reasons that might explain why some of these bulls sired exceptionally high producing daughters while others transmitted only a low level of production.

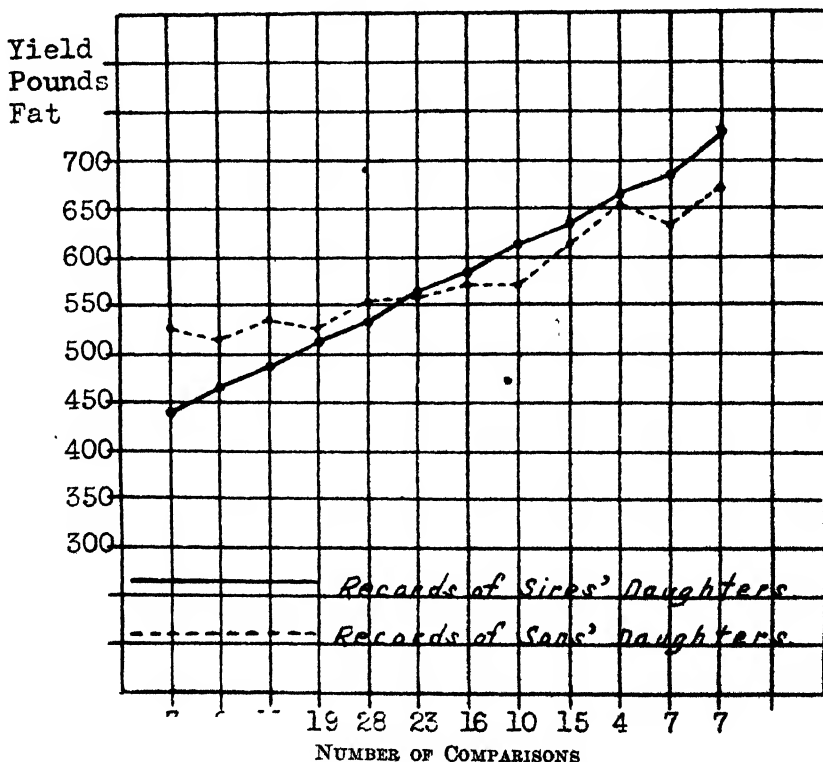


FIG. 1. Comparison of records of the daughters of "Tested Sires" with the records of their sons' daughters.

There are 156 bulls with ten or more tested daughters that in turn have sons with ten or more tested daughters. The relationship or correlation between the records of the sires' daughters and the records of the sons' daughters should furnish an indication of the value of the records of the sires' daughters in measuring the transmitting ability of the son. The results of this comparison are portrayed in Table 1 and Figure 1.

Of the 729 sires, 385 were out of tested dams. Again, the relationship between the dams' records and the sons' daughters' production will indicate the value of a production record in evaluating the transmitting ability of the dam. In other words, the records of these 385 dams were compared with the average of the records of their sons' daughters. The results of this comparison are shown in Table 1 and Figure 2.

Examination of this table and the accompanying figures reveals that the coefficient of correlation between the records of the sires' daughters and the records of the sons' daughters is very good, while the correlation between the dams' own records and the sons' daughters' records is only fair. These results are significant and demonstrate that in the selection of a bull calf it is more important to consider the average production of his sire's daugh-

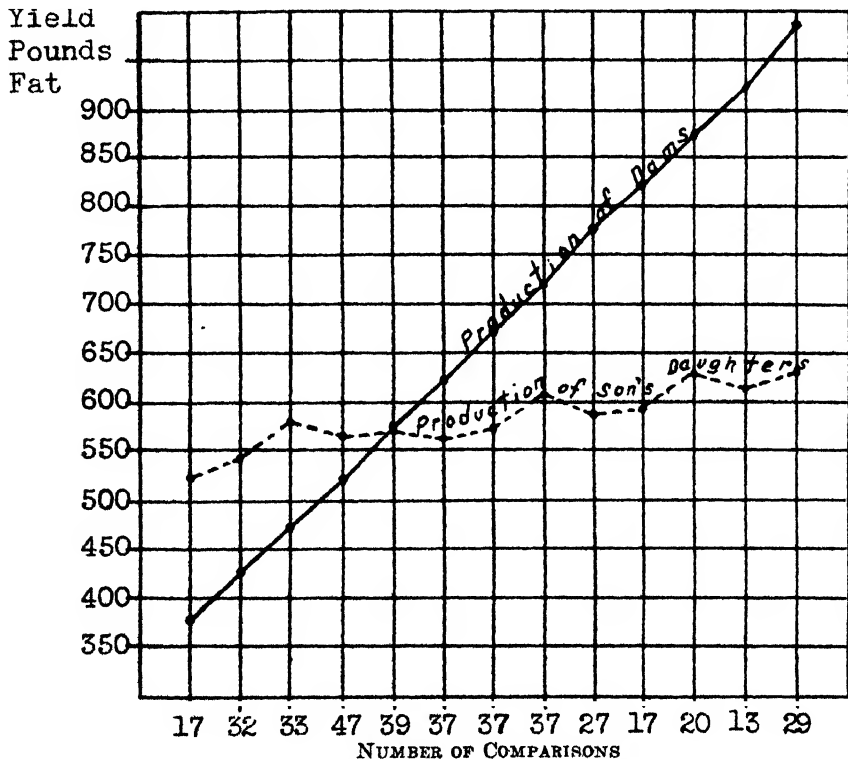


FIG. 2. Comparison between records of dams and records of their sons' daughters.

ters than it is to limit consideration to his dam's record alone. The correlation between the dams' records and sons' daughters' records compares favorably with that previously reported by the author (5). Gowen (6) working with Holsteins found a slightly less correlation ($+0.307 \pm 0.047$) between maternal grandams and granddaughters. It is to be noted that the genotypes of the bulls have been compared to the genotypes of their sires but to the phenotypes of their dams.

TABLE 1
Physical constants for sires' and sons' daughters and dams and sons' daughters

	MEAN	STANDARD DEVIATION	CORRELATION BETWEEN SIRE'S DAUGHTERS AND AND SONS' DAUGHTERS	CORRELATION BETWEEN DAMS' RECORDS AND SONS' DAUGH- TERS
Sires .. .	565.16 ± 3.9	72.14 ± 2.7	+ 0.558 ± .055	+ 0.331 ± .031
Sons' daughters	572.21 ± 3.6	65.74 ± 2.5		
Dams ...	651.72 ± 5.9	172.61 ± 4.1		
Sons' daughters	581.88 ± 2.8	79.92 ± 1.9		

The author's attention has been called to the possibility of the range in production of a bull's daughters furnishing valuable information in regard to his transmitting ability. In studying the records made by the progeny of a bull, is it important to know what proportion of his daughters are above the breed average and what proportion are inferior? To study this phase of the question the group of 156 sires having "Tested" sons was used. The daughters of each sire were segregated in various groups, depending on their butterfat yield. Then the percentages of daughters over 500 pounds of fat, over 550 pounds, over 600 pounds and over 650 pounds were determined for each bull. These percentages were next compared with the average yield of the sons' daughters. The results are given in Table 2. It will be observed that the percentage of a bull's daughters over 600 pounds of fat is just about equal to but not any better than the average production of all his tested daughters in estimating the production of the sons' daughters.

There were 312 sires with ten or more tested daughters that in turn had paternal grandsires with ten or more tested daughters. The average yields of the paternal grandsires' daughters were compared with the average yields of the grandsons' daughters. This was done in order to estimate the amount of consideration that should be given to the paternal grandsire in pedigree study. Similarly, it was found that 241 of the bulls listed had maternal grandsires with ten or more tested daughters. A like comparison was made between the average yield of the maternal grandsires' daughters

TABLE 2
Correlation coefficients of ranking of sires' daughters and yield of sons' daughters

	MEAN	CORRELATION BETWEEN PERCENTAGES OF SIRE'S DAUGHTERS OVER 500 LBS., OVER 550, 600 AND 650 LBS. AND YIELD OF SONS' DAUGHTERS
Percentage of sires' daughters over 500 lbs.	64.3 \pm 1.25	+ 0.434 \pm .044
Percentage of sires' daughters over 550 lbs.	48.9 \pm 1.28	+ 0.496 \pm .041
Percentage of sires' daughters over 600 lbs.	35.1 \pm 1.24	+ 0.550 \pm .038
Percentage of sires' daughters over 650 lbs.	24.7 \pm 1.11	+ 0.528 \pm .038
Yield of sons' daughters	572.2 \pm 3.6	

and the grandsons' daughters. The results of these comparisons given in Table 3 show a significant difference between the correlation figures to the two grandsires.

TABLE 3
*Physical constants for paternal and maternal grandsires' daughters and
 grandsons' daughters*

	MEAN	STANDARD DEVIATION	CORRELATION BETWEEN GRANDSIRE'S DAUGHTERS AND GRANDSON'S DAUGHTERS
Paternal grandsires' daughters	534.05 \pm 2.3	60.45 \pm 1.6	+ 0.250 \pm .036
Grandsons' daughters	576.52 \pm 2.7	71.76 \pm 1.9	
Maternal grandsires' daughters	554.18 \pm 3.2	72.72 \pm 2.2	+ 0.427 \pm .036
Grandsons' daughters	577.54 \pm 3.3	75.60 \pm 2.3	

The possibility that some of the factors governing the inheritance of milk yield may be inherited in a sex-linked manner has been suggested previously by Smith, Scott & Fowler (7) and Madsen (8). Gowen (9), however, believes that the sire and the dam are equally and jointly responsible for the milk production and butterfat percentages of their offspring, also Fohrman and Graves (10) doubt the assumption of sex-linkage in the inheritance of milk yield.

An interesting side-light on this phase of the problem was accidentally encountered in looking up information on the bull, Pogis 99th of Hood Farm. A pronounced difference was observed in the production of the

grandsons' daughters where Pogis 99th of Hood Farm occurred as the maternal grandsire instead of the paternal grandsire. In checking through the "Tested Sire" booklet it was found that of the bulls whose daughters averaged over 600 pounds of fat there were just three that had five or more "tested" grandsons sired by sons and five or more "tested" grandsons out of daughters. The names of these three bulls are Pogis 99th of Hood Farm 94502, Fauvic's Prince 107961 and Hood Farm Torono 60326. The data on these sires is shown in table 4.

In all three of these examples it would appear that where the bulls named occurred as the maternal grandsires they transmitted the factors of production on to their grandsons to a slightly greater extent than they did when they appeared as the paternal grandsires.

TABLE 4

NAME OF GRANDSIRE	NO. OF DAUGHTERS	AVER. YIELD OF DAUGHTERS	NO. OF PATERNAL GRANDSONS	AVER. YIELD OF DAUGHTERS OF PATERNAL GRANDSONS	NO. OF MATERNAL GRANDSONS	AVER. YIELD OF DAUGHTERS OF MATERNAL GRANDSONS
P 99 of H. F.	119	693.88	12	580.86	10	619.04
F. P.	62	728.08	5	557.52	5	604.22
H. F. T.	72	637.34	14	581.03	22	600.57

These results might be explained as indicating a sex-linked inheritance and it would appear not unreasonable to assume that at least a few of the factors affecting the inheritance of milk production may be transmitted on the sex chromosome. On the other hand it will be noted that the dams of the tested sires were a highly selected group having an average butterfat yield considerably above the breed average. Furthermore, as shown further on, the daughters of these dams are considerably above the breed average in producing ability. In other words, it is possible that the daughters of the maternal grandsires were a more highly selected group than were the sons of the paternal grandsires. It may be that this explanation will account for all of the difference between the correlation to the paternal grandsires' daughters and the maternal grandsires' daughters. It is to be hoped that further work will be undertaken in connection with the other breeds to present further evidence on this important question.

Through the progeny test we have a very good means of predicting a bull's potential transmitting ability. Similarly, the best index of the germinal composition of the dam would be her transmitting ability of desired characters to her daughters. This index is not available to the same extent that it is in the case of bulls. Very few cows have as many as even 5 tested daughters. It was found that there were 51 bulls listed that were out of

cows having three or more tested daughters. The records of the daughters of these dams were averaged and the averages of their production were compared to the average productions of the sons' daughters. Ninety bulls listed were out of dams having two tested daughters and 172 of the bulls were out of dams having one tested daughter. Similar comparisons were made between the averages of these dams' daughters and the averages of the sons' daughters. The results are pictured in table 4. Where the dam has three or more tested daughters, the amount of correlation is particularly good. In fact the record of only one tested daughter seems to be equal to the dam's own record in rating her transmitting ability.

TABLE 5
Coefficients of correlation of dams' daughters and sons' daughters

	MEAN	STANDARD DEVIATION	CORRELATION BETWEEN DAMS' DAUGHTERS AND SONS' DAUGHTERS
Dams with three or more tested daughters	615.61 \pm 10.50	111.16 \pm 7.43	+ 0.466 \pm .074
Sons' daughters	593.67 \pm 7.45		
Dams with two tested daughters	574.44 \pm 6.07	85.45 \pm 4.30	+ 0.378 \pm .061
Sons' daughters	594.58 \pm 5.56	78.27 \pm 3.94	
Dams with one tested daughter	600.06 \pm 7.76	150.63 \pm 5.49	+ 0.357 \pm .045
Sons' daughters	581.86 \pm 3.99	77.44 \pm 2.82	

The average production of all (9265) 365 day Register of Merit records ever completed by mature cows from 5 to 10 years of age, inclusive, is 549.22 pounds of butterfat. This, it appears, may be reasonably considered as the mature average 365 day production of the breed for cows tested in the Register of Merit. The average production of the daughters of the 729 "Tested" sires computed to maturity is 557.71 pounds of fat. Thus the daughters of the 729 sires listed were slightly better than the breed average although in the list there were 162 bulls whose daughters averaged less than 500 pounds of fat and of these 42 had daughters averaging less than 450 pounds. Such bulls are apparently heterozygous in their genetic make-up. On the other hand, the germinal composition of the bulls whose daughters average over 600 pounds of fat should be quite homozygous. If this is so, an examination of the transmitting ability of such sires should give valuable results.

Of the 156 bulls with "Tested" sons, the daughters of only 43 average above 600 pounds of fat. Taking these 43 sires for study, it was found that 25 (58.14%) had sons whose daughters in turn average above 600 pounds of fat. Of the 385 cows with "Tested" sons, 217 have records higher than 600 pounds of butterfat. The sons of 95 (43.78%) of these cows in turn sired daughters averaging above 600 pounds of fat.

There were 42 cases in which the daughters of paternal grandsires of "Tested" sires averaged over 600 pounds of fat. Twenty (47.62%) of the grandsons of these 42 bulls in turn sired daughters averaging over 600 pounds. Examination of the list of "Tested" sires having "Tested" maternal grandsires revealed that there were 59 of such maternal grandsires whose daughters average over 600 pounds of fat. Thirty-four (57.63%) of their grandsons sired daughters averaging over 600 pounds of fat. Grouping the dams with three or more tested daughters and those with two tested daughters together gave a total of 54 dams with two or more tested daughters averaging over 600 pounds of fat. Thirty-

TABLE 6
Tabulation of sires with high producing and high transmitting ancestry.

	TOTAL NUMBER	NUMBER OVER 600 LBS. FAT	NUMBER OF SONS OR GRANDSONS WITH DAUGHTERS AV. 600 LB. FAT	PERCENTAGE OF SONS OR GRAND- SONS WITH DAUGHTERS AVERAGING OVER 600 LBS. FAT
"Tested" bulls with "Tested" sons	156	43	25	58.14%
Record cows with "Tested" sons	385	217	95	43.78%
"Tested" paternal grandsires with "Tested" grandsons	312	42	20	47.62%
"Tested" maternal grandsires with tested grandsons	241	59	34	57.63%
Dams with 2 or more record daughters and having "Tested" sons	141	54	31	57.41%
"Tested" bulls by "Tested" sires whose daughters av. over 600 lbs. and whose dams have 2 or more daughters averaging over 600 lbs.		27	21	77.78%
"Tested" bulls by "Tested" sires whose daughters av. over 600 lbs., whose dams have 2 or more daughters averaging over 600 lbs. and whose maternal grandsires 10 or more record daughters av. 600 lbs.		14	12	85.71%

one (57.41%) of the sons of these dams sired progeny averaging over 600 pounds of fat. These results compare favorably with the correlation data and indicate that in studying a pedigree more weight should be given to the sire's daughters, dam's daughters and maternal grandsire's daughters than to the dam's record or the paternal grandsire's daughters. Another reason that the paternal grandsire's daughters need not be given so much importance is that the records of the sire's daughters represent the inheritance from the sire's side.

"Tested" sires out of dams having two or more daughters averaging over

Following on this line of work, it was found that there were 27 600 pounds of butterfat and in turn sired by bulls whose daughters all average about 600 pounds of fat. Twenty-one, or 77.78% of these bulls sired daughters, the average of whose production exceeded 600 pounds of fat. Going back still further in the pedigree, it was ascertained that there were just 14 bulls whose sires' daughters, dams' daughters and maternal grandsires' daughters all average over 600 pounds of fat. Twelve of these bulls, or 85.71%, in turn sired daughters averaging over 600 pounds of fat and in the other two instances the daughters averaged considerably over 500 pounds of butterfat. The accompanying Table 5 illustrates these results.

SUMMARY AND CONCLUSIONS

1. In studying the ancestry of a bull calf the records of the sire's daughters are considerably more valuable than is the record of the dam alone.

2. On the evidence submitted, the records of the daughters of the maternal grandsire are more closely related to the production of the grandsons' daughters than are the records of the daughters of the paternal grandsire.

3. It would appear that if a cow has a production record herself and if she has two or more tested daughters and if in turn her sire has a number of tested daughters, the sum of this information gives a good index of her germinal composition. This combined information is necessary to accurately evaluate a cow's transmitting ability.

4. The selection of a calf with a pedigree as follows should reduce to a minimum the element of chance involved. A large majority of young bulls with such pedigrees should sire high producing daughters, particularly if bred to cows possessing an average inheritance of production.

Prospective bull calf	Sire	Paternal Grandsire
	Ten or more tested daughters averaging over 600 lbs. of fat.*	If possible 10 or more tested daughters averaging over 600 lbs. although not absolutely essential.
		Paternal Grandam
	Dam	Maternal Grandsire
	Individual record over 600 lbs.* Two or more tested daughters averaging over 600 lbs.*	If possible 10 or more tested daughters averaging over 600 lbs. of fat.*
		Maternal Grandam

* All records computed to a mature 365 day basis.

REFERENCES

- (1) GIFFORD, WARREN. The Mode of Inheritance of Yearly Butterfat Production. Mo. Agr. Exp. Station Research Bulletin 144. 1930.
- (2) and (6) GOWEN, JOHN W. Milk Secretion. Williams and Wilkins, Publishers. 1924.
- (3) COPELAND, LYNN. What is Required to Prove a Bull? Jersey Bulletin, January 6, 1932.
- (4) EDWARDS, JOSEPH. The Progeny Test as a Method of Evaluating the Dairy Sire. Journal of Agricultural Science, 22: Part IV, October, 1932.
- (5) COPELAND, LYNN. The Contribution of the Dam in Inheritance of Milk and Butterfat. JOURNAL OF DAIRY SCIENCE, 14: 5, September, 1931.
- (7) SMITH, A. D. BUCHANAN, SCOTT, R. J., and FOWLER, A. B. The Inheritance of Milk Yield in Ayrshire Cows. Journal of Dairy Research, 1: No. 2, May, 1930.
- (8) MADSEN, KARL. Inheritance of Milking Capacity. Nature, January 30, 1932.
- (9) GOWEN, JOHN W. Milk Secretion as Influenced by Inheritance. The Quarterly Review of Biology, 11: No. 4, December, 1927.
- (10) FOHRMAN, M. H., and GRAVES, R. R. Analysis of the Advanced Registry Records of 611 Daughters of 51 Ayrshire Sires. U. S. Dept. of Agr., Technical Bulletin 349, March, 1933.

THE MANUFACTURE OF LOW-LACTOSE SKIM MILK FOR USE IN ICE CREAM

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The percentage of milk-solids-not-fat ordinarily used in ice cream mixes has been limited by the amount of lactose which could be incorporated in a mix without any great possibility of the development of sandiness. A method by which the percentage of milk proteins could be increased without increasing the percentage of lactose in the mix beyond that which causes sandiness, would be of value in improving the body and texture of ice cream, in safeguarding the product against possible crystallization of the lactose, and in increasing the consumption of milk solids.

One patented method (1) of increasing the milk solids of an ice cream mix consists in the addition of milk powder to the mix during freezing, but such a procedure also incorporates sufficient lactose to produce a super-saturated solution of this sugar in cases where the frozen product is partially melted or subjected to heat-shocking and subsequent freezing. Another method involves the addition of any one of several soluble milk proteins found on the market. These proteins are not in the state in which they normally occur in skim milk; they have been removed by chemical means and redispersed, in most cases in weakly-alkaline solutions.

The experimental work reported in this paper was undertaken with the object of perfecting a method for the removal of a large percentage of the lactose from skim milk without changing the normal dispersion of its proteins. Experiments showing the advantages of this low-lactose skim milk (for convenience this product is called "low-lac") in ice cream have been carried out.

The basic data, upon which the process for manufacturing this low-lactose milk rests, have been reported by Leighton and Leviton of these laboratories (2). These investigators have shown "that the addition of cane sugar in suitable amounts to skim milk before condensing markedly lowers the viscosity of the highly concentrated product," and that "this phenomenon can be attributed to the diluting action of the cane sugar on the basis that the dissolved sugar markedly increases the volume of the liquid phase of the suspension." Leighton and Leviton have also shown that the cane sugar inhibits an increase in the viscosity of the concentrated milk on standing, and furthermore that, by means of this addition of cane sugar to skim milk before condensing, the viscosity of the concentrated product is reduced "to the point where lactose crystallization can take

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place readily and where separation of the milk sugar crystals by mechanical means is feasible."

MANUFACTURE OF LOW-LAC MILK

The optimum quantity of cane sugar to add to 100 pounds of skim milk for maintenance of minimum viscosity in the concentrated product has been shown by Leighton and Leviton to be 5.9 pounds. The mixture should be forewarmed to 65° C. for 10 minutes before it is drawn into the vacuum pan, where a concentration of 68 to 70 per cent total solids is attained. As an aid in striking the batch a striking cup may be used. The specific gravity of the finished milk at 40° C. should be between 1.32 and 1.34, and its viscosity at this temperature will range between 26 and 32 centipoises (2). The refractive index of the condensed product at 40° C. should be approximately 1.470 to 1.490. After it is drawn from the pan the concentrated milk is next cooled to between 20° and 25° C. in cold water with occasional slow stirring. It is then held about 20 hours in an ice box at 10° C.; this temperature being preferred to a higher temperature (where maximum crystallization would occur) in order to prevent the development of excessive viscosity, a characteristic of milks held at high storage temperatures. During this time the lactose crystallizes and can be removed from the milk by means of a basket centrifuge, or preferably by means of a filter press.

The separation of the lactose crystals is the only step in the process which offers any serious obstacle to the preparation of low-lac by any dairy plant equipped with a vacuum pan. The milk sugar forms small crystals, the removal of which from the heavy condensed milk renders special equipment necessary. When the viscosity of the product is not excessively great, filter pressing is the most rapid means of separating the lactose. For all viscosity conditions and crystal sizes, centrifugal separation is very satisfactory. For either centrifuge or filter press the filter cloths which have been found satisfactory are of very fine mesh.

The quantity of lactose which can be removed from the condensed milk will vary from 40 to 75 per cent of the total lactose present in the milk. Under favorable conditions an average of 60 to 70 per cent can be removed.

There is less tendency toward bacterial spoilage in low-lac than in plain condensed skim milk because of the added cane sugar. The parts of cane sugar to 100 parts of water (sugar ratio) will vary in low-lac from 45 to 50 which is considerably below the required figure of 63 to 65 necessary to prevent bacterial growth. Additional cane sugar may be added but a very heavy bodied milk will result.

The viscosity of low-lac increases during storage, a high viscosity developing more rapidly where the temperature of storage is high. For routine production it may be found desirable to dilute the finished product with plain skim milk to attain a uniform concentration of about 50 per cent total

solids. By thinning the milk in this manner the inconvenience of handling a heavy bodied product will be avoided.

Approximately 50 different batches of low-lac have been made in these laboratories, using about 200 pounds of skim milk per batch. The method of manufacture has also been found satisfactory when tried out on a semi-commercial scale at the Grove City Creamery, Grove City, Pa., where batches of approximately 1,400 pounds of skim milk were converted into low-lac.

The cost of manufacture of low-lac above that of normal condensed milk lies only in the labor necessary to remove the lactose. The additional labor consists of two steps: (1) Dropping the condensed milk from the condenser into crystallizing cans where it can be cooled and held over night, and (2) separating the crystals from the milk. The lactose thus obtained may be washed and marketed as crude lactose, or it may be recrystallized to prepare a relatively pure sugar. The expense for the above operations will vary with local conditions but it seems probable that the market value of the lactose would, under normal circumstances, cover the cost of separating it. It should be possible to obtain from 2 to 2½ pounds of washed crude lactose per 100 pounds of skim milk.

In table 1 the data from a number of representative batches of milk are reproduced. Determinations of total solids were made on each condensed milk before and after removal of the lactose. From these figures the percentage of lactose removed was calculated.

TABLE 1

Showing the total solids of representative batches of skim milk with the calculated percentage of lactose removed

BATCH NO.	TOTAL SOLIDS		LACTOSE REMOVED
	Condensed, after evaporation	Low-lac after re- moval of lactose	
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	67.70	63.12	57.8
2	71.42	66.06	69.8
3	67.10	62.91	52.8
4	71.42	66.70	62.1
5	71.09	65.33	74.4
6	70.74	64.40	79.0
7	71.18	67.12	54.6
8	69.31	63.41	72.7
9	74.91	69.99	67.8

Average percentage lactose removed 65.6

CALCULATIONS INVOLVED IN THE MANUFACTURE AND USE OF LOW-LAC

The calculations necessary to properly control the composition of an ice cream mix when low-lac is used are somewhat involved. Removal of part

of the lactose from the milk alters the ratio of lactose to other milk solids. This altered ratio together with the percentage of lactose desired in a mix must be considered in calculating the quantities of the other ingredients. The percentage of lactose separated from the skim milk is easily calculated using the following formula:

$$1 - \frac{f(100-c)}{c(100-f)} \times 100 \quad k = \% \text{ lactose removed}$$

where: f = per cent total solids of low-lac filtrate
 c = per cent total solids of condensed skim milk
 k = a constant which changes only with the composition of fresh skim milk

For accurate results the composition of the fresh skim milk should be ascertained by analysis, but for the purpose of this work satisfactory figures were secured by considering the average composition of skim milk to be (3) as follows:

Water	90.35%
Protein	3.27%
Fat	0.15%
Lactose	4.98%
Ash	0.80%

Using these figures together with the quantities of fresh skim milk and cane sugar used in the batch the components of k become:

$$\begin{aligned} .0498 &= \text{lactose in 1 part fresh skim milk} \\ .0965 &= \text{total solids in 1 part skim milk} \\ 160 &= \text{lbs. milk used} \\ 9.5 &= \text{lbs. sugar used} \\ \text{and } k &= \frac{(.0965 \times 160) + 9.5}{.0498 \times 160} = 3.13 \end{aligned}$$

Assuming a batch of low-lac is made, and that the condensed skim milk before removal of the lactose tested 71.4 per cent total solids and the low-lac tests 66.0 per cent total solids then:

$$\text{Percentage lactose removed} = 1 - \frac{66.0(100-71.4)}{71.4(100-66.0)} \times 313 = 69.79\%$$

It is necessary to know the composition of the low-lac in order to be able to calculate the ice cream mixes. The following example illustrates the method used to obtain these figures.

Since 9.5 pounds cane sugar was added to 160 pounds skim milk, the total weight of the material before condensing was 169.5 pounds. The percentage of cane sugar was then $\frac{9.5}{169.5} \times 100 = 5.60$. Percentages of the

various constituents in the condensed milk may be found by substitution in the proportion:

Percentage total solids in sweetened skim : percentage total solids in condensed : : percentage a in sweetened skim : percentage a in condensed, or $\frac{100}{105.6} (9.68) + 5.60 : 71.4 : : \frac{100}{105.6} (\% a \text{ in skim}) : \% a \text{ in condensed}$

From this, $4.58 \times \% a \text{ in skim} = \% a \text{ in condensed}$, except for the cane sugar, for which $4.84 \times \% \text{ cane sugar in sweetened skim} = \% \text{ cane sugar in condensed}$.

Whence, in the condensed:

Protein plus ash	20.84%
Lactose	22.81%
Cane sugar	27.09%
Fat	0.69%
Total solids	71.43%

Of the lactose in the condensed, 69.8% of 22.81, or 15.92, is the proportion removed, 6.89 remaining. The low-lac is then $100 - 15.92 = 84.08\%$ of the condensed. Hence the composition of the low-lac is the following:

$$\begin{aligned} \frac{20.84}{0.8408} &= 24.78\% \text{ protein plus ash} \\ \frac{6.89}{0.8408} &= 8.20\% \text{ lactose} \\ \frac{27.09}{0.8408} &= 32.22\% \text{ cane sugar} \\ \frac{0.69}{0.8408} &= 0.82\% \text{ fat} \\ &\underline{\hspace{1.5cm}} \\ &66.02\% \text{ total solids} \end{aligned}$$

Low-lac should be used in an ice cream mix only when there is likelihood that the lactose of normal skim milk, upon further addition of this product, may cause sandiness. Low-lac is intended only to supplement the milk products which normally supply the serum solids of a mix. For example, a mix of 10 per cent milk-solids-not-fat should be made without low-lac. However, a mix of 12 per cent milk-solids-not-fat should derive about 9 per cent of its milk solids from the usual sources (cream, and skim milk) and 3 per cent from low-lac.

There are several methods by which ice cream mixes can be calculated when low-lactose milk is to be used. The method given below appears to be the most simple since it makes use of the familiar square method of standardization. It should here be borne in mind that although an appreciable amount of calculation may be necessary in the manufacture and use of the

first few batches of low-lac, considerable standardization can be worked out which will eliminate most of the calculations.

In calculating a mix it is necessary first to ascertain the percentage of milk-solids-not-fat to be derived from products having a normal lactose content such as skim milk and cream and also that percentage of solids-not-fat to be added in the form of low-lac. Table 2 gives the percentage of lactose which will normally be present in mixes of the designated milk-solids-not-fat percentages. A mix in which the lactose exceeds 5.24 to 5.50 per cent, corresponding to a milk-solids-not-fat content of 10.0 to 10.5 per cent will generally be susceptible to the development of sandiness.

TABLE 2

Showing the percentage of lactose in mixes having a normal ratio of M.S.N.F. to lactose

(% Lactose = $\frac{\text{M.S.N.F.}}{1.908}$ when lactose is present in the ratio of 4.98 per cent to 9.50 per cent milk-solids not-fat)

% M.S.N.F.	% Lactose
10.0	5.24
10.5	5.50
11.0	5.77
11.5	6.03
12.0	6.29
12.5	6.55
13.0	6.82

As an example of the method of calculation consider the composition of the low-lactose milk and of the ice cream mix to be as follows:

Milk constituent	Desired mix composition %	Composition of low-lac %
Fat	12.0	.82
M.S.N.F.	12.0	32.98
Cane sugar	14.0	32.22
Gelatin	0.3	
T.S.	38.3	66.02

Let the limiting lactose content of the mix be 5.24 per cent. With a normal source of milk solids a mix of 12.0 per cent S.N.F. would contain 6.29 per cent lactose.

If the entire 12.0 per cent of solids-not-fat was made up of this low-lac then in the following proportion x = the percentage which would be lactose:

$$32.98 : 8.20 :: 12 : x$$

$$x = 2.99$$

Now the familiar standardization square may be used to ascertain the proportion of lactose which is to be obtained from normal milk solids and from the lactose-free skim milk.

2.99		1.05 = parts lactose from low lac
	desired % lactose 5.24	
6.29		2.25 = parts lactose from normal milk
		3.30 = total parts

Converting parts lactose to per cent milk-solids-not-fat :

$$\frac{2.25}{3.30} \times 12 = 8.18 \% \text{ S.N.F. to be obtained from normal condensed milk}$$

and cream.

$$\frac{1.05}{3.30} \times 12 = 3.82 \% \text{ S.N.F. to be obtained from low-lac}$$

Therefore :

$$\frac{\% \text{ S.N.F. from low-lac}}{\% \text{ S.N.F. in low-lac}} \times \text{lbs. mix desired} = \text{lbs. low-lac to be used}$$

$$\frac{3.82}{32.98} \times 100 = 11.58 \text{ lbs. low-lac for 100 lbs. of this mix}$$

With this quantity of low-lac there will also be added

$$11.58 \times 32.22 \div 100 = 3.73 \text{ lbs. cane sugar}$$

$$11.58 \times .82 \div 100 = .95 \text{ lbs. fat}$$

For 100 pounds of the original mix there now remain the following weights of ingredients to be secured and added in the usual way :

M.S.N.F.	= 12.00 - 3.82 = 8.18 per cent
Fat	= 12.00 - .95 = 11.05 " "
Sugar	= 14.00 - 3.73 = 10.27 " "
Gelatin	0.30 " "

Calculations, according to the method given above, have been made over a wide range of lactose concentrations and the results obtained are given in table 3. By means of this table the lengthy calculations which it is necessary to make when low-lac is used in a mix may be dispensed with. While the figures given are not strictly accurate they will be found to be sufficiently exact in most cases.

To use table 3 it is first necessary to determine, within one tenth of one per cent, the percentage of total solids both in the condensed milk, before the lactose is removed, and in the resultant low-lac milk. Having obtained these figures subtract the percentage of the total solids in the low-lac milk from the percentage of total solids in the condensed milk. A figure representing this difference will be found in the first vertical column of the table.

TABLE 3

Showing the percentage of lactose removed from condensed skim milk when the percentage of its total solids is lowered over a range of 4.0 to 6.0 and the amount of the resultant low-lac milk to add 100 pounds of ice-cream mix in order to raise the percentage of M.S.N.F. from 10 to 11. (The pounds of cane sugar and of M.S.N.F. in the specified quantity of added low-lac milk are also given)

TOTAL SOLIDS OF CONDENSED MILK MINUS TOTAL SOLIDS OF LOW-LAC MILK	Per cent	WHEN THE PERCENTAGE OF TOTAL SOLIDS OF THE CONDENSED MILK, BEFORE REMOVAL OF THE LACTOSE WAS—					
		62	64	66	68	70	72
4.0	Lactose removed from condensed milk—per cent	47.5	48.7	49.3	50.6	52.4	53.7
	Low-lac milk to add to ice-cream mix—pounds	10.55	9.96	9.45	8.90	8.21	7.76
	Cane sugar in the added low-lac milk—pounds	2.77	2.72	2.67	2.61	2.50	2.45
	M.S.N.F. in the added low-lac milk—pounds	3.35	3.25	3.18	3.08	2.91	2.83
4.5	Lactose removed from condensed milk—per cent	53.1	53.7	55.3	56.2	57.7	59.9
	Low-lac milk to add to ice-cream mix—pounds	9.28	8.86	8.30	7.87	7.38	6.86
	Cane sugar in the added low-lac milk—pounds	2.47	2.44	2.38	2.34	2.28	2.20
	M.S.N.F. in the added low-lac milk—pounds	2.87	2.83	2.72	2.66	2.56	2.43
5.0	Lactose removed from condensed milk—per cent	58.3	59.3	60.5	61.4	63.3	65.5
	Low-lac milk to add to ice-cream mix—pounds	8.37	7.98	7.46	7.12	6.63	6.16
	Cane sugar in the added low-lac milk—pounds	2.25	2.23	2.17	2.14	2.08	2.01
	M.S.N.F. in the added low-lac milk—pounds	2.52	2.48	2.38	2.34	2.23	2.12
5.5	Lactose removed from condensed milk—per cent	63.3	64.3	65.6	67.1	68.8	70.8
	Low-lac milk to add to ice-cream mix—pounds	7.64	7.25	6.82	6.43	6.00	5.62
	Cane sugar in the added low-lac milk—pounds	2.08	2.05	2.01	1.97	1.91	1.86
	M.S.N.F. in the added low-lac milk—pounds	2.24	2.19	2.12	2.05	1.96	1.88
6.0	Lactose removed from condensed milk—per cent	68.3	69.2	70.8	72.3	73.8	76.1
	Low-lac milk to add to ice-cream mix—pounds	6.97	6.67	6.25	5.88	5.52	5.16
	Cane sugar in the added low-lac milk—pounds	1.92	1.91	1.87	1.83	1.78	1.73
	M.S.N.F. in the added low-lac milk—pounds	1.99	1.96	1.89	1.82	1.76	1.67

All calculations are based on the average composition of skim milk as given in *Fundamentals of Dairy Science*, by Associates of Rogers, as follows: Protein, 3.72 per cent; lactose, 4.98 per cent; and ash, .80 per cent; total solids, 9.50 per cent. The 0.15 per cent fat has been disregarded.

In cases where the actual values do not fall near those in the table interpolation of the figures in the table may be made.

In each square in table 3, corresponding to the various percentages of lactose removed from milks differing in their original concentration, are found four figures. These are: (1) the percentage of lactose which has been removed from the condensed milk (this figure is given as a matter of information only and is not to be used in figuring mix from this table); (2) the pounds of low-lac milk to be added per 100 pounds of mix to raise the percentage of M.S.N.F. from 10 to 11 (10 per cent M.S.N.F. can be safely attained without the use of low-lac); (3) and (4) the pounds of cane sugar and of M.S.N.F. respectively, which are contained in the low-lac added to the mix. The quantity of cane sugar and of M.S.N.F. thus added to the mix must be taken into consideration in making up the rest of the mix.

To raise the percentage of M.S.N.F. from 10 to 11, use the figures direct from the table, but to raise the percentage of M.S.N.F. to 12 or 13 multiply the weights of ingredients given in the table by 2 or 3 as the case may be.

The following example will serve to illustrate how table 3 may be used in making the calculations for 100 pounds of mix containing 11 per cent M.S.N.F.

Suppose your determinations have shown the total solids in the original condensed milk to be 68.0 per cent, and the total solids in the resultant low-lac milk to be 63.0 per cent. The difference is 5.0 per cent.

Find the figure 5 in the first vertical column of the table and read across to the vertical column that represents the 68 per cent total solids in the original condensed milk. It will be found that 7.12 pounds of low-lac milk should be added per 100 pounds of mix to raise the percentage of M.S.N.F. from 10 to 11.

Also, it will be found that the 7.12 pounds of low-lac milk contains 2.14 pounds of cane sugar and 2.34 pounds of M.S.N.F.

Therefore, in making up a mix that is to contain 14 pounds of cane sugar it will be necessary to add $14 - 2.14$, or 11.86 pounds of additional sugar. Similarly, if the mix is to contain 12 pounds of M.S.N.F., there must be added $12 - 2.34$, or 9.66 pounds of additional M.S.N.F., which may be obtained from other sources such as condensed milk, skim milk, milk powder, or cream.

Should the values for total solids not fall within 0.1 to 0.2 per cent of those given in the table, a satisfactory approximation may be obtained as follows:

Suppose your determinations have shown the total solids in the original condensed milk to be 68.8 per cent, and the total solids in the resultant low-lac milk to be 64.1 per cent. The difference is 4.7 per cent.

Since there are not constants in table 3 comparable to the 68.8 per cent

Comparative data on ice-cream mixes made with and without low-lac

MIX NUMBER	FAT	COMPOSITION OF MIX*						FREEZING DATA				PLACINGS BASED ON NUMERICAL SCORES IN ORDER OF PREFERENCE			
		M.S.N.F.			Sugar			Freezing time	Maxi- mum over- run	Final run	Flavor	Texture	Total		
		Plain cond.	Source		Total	Cane	Lactose								
			Cond.	Powder											
														%	%
Expt. No. 1: Mix 1 Mix 2	12 12 12	12.0 7.82	4.18	12 12	14 14	10 11 12 13	14 14 14 14	5.24 5.24 5.24 5.24	14 16 21 19	86 88 90 102	93 93 89	93 89	2-1		
Expt. No. 2: Mix 1 Mix 2 Mix 3 Mix 4	12 12 12 12	10.0 7.67 5.33 2.7		3.33 6.67 10.3	10 11 12 13	14 14 14 14	5.24 5.24 5.24 5.24	14 16 21 19	86 88 90 102	86 84 85 82	1-2-3-4 1-(2-3)-4 **	1-2-3-4	1-2-3-4		
Expt. No. 3: Mix 1 Mix 2 Mix 3 Mix 4	12 12 12 12	10.0 7.67 5.33 2.74		3.33 6.67 10.3	10 11 12 13	14 14 14 14	5.24 5.24 5.24 5.24	20 23 25 27	99 113 117 121	85 84 85 85	(1-2)-3-4 4-(2-3)-1	2-3-1-4	2-3-1-4		
Expt. No. 4: Mix 1 Mix 2 Mix 3	12 12 12	10.0 1.25 0.19	10.75 13.81	10 12 14	14 14 14	5.55 5.20 5.09	14 16 13	83 103 106	83 80 82	1-2-3	2-1-3	2-1-3	2-1-3		
Expt. No. 5: Mix 1 Mix 2 Mix 3	12 10 8	10.0 1.46 0.71	10.54 13.29	10 12 14	14 14 14	5.55 5.13 5.64	16 16 17	95 106 112	80 80 82	(1-2)-3	2-1-3	2-1-3	2-1-3		
Expt. No. 6: Mix 1 Mix 2 Mix 3	12 12 12	1.2 0.97	10.8 11.03 14	12 12 14	14 16 14	5.17 4.99 4.92	19 16 14	114 102 104	81 81 82	1-2-3	2-1-3	2-1-3	2-1-3		
Expt. No. 7: Mix 1*** Mix 2	12 12	12.0 10.0	2	12 12	14 14	6.29 5.24				1-2	1-3	1-2	1-2		

* All mixes contained 0.3% gelatin.

** Parentheses indicate tie score.

*** Developed sandiness on heat-shocking after being scored.

and the 4.7 per cent, the rule to follow is to use the nearest higher figure to the 68.0, or 70 in this case, and the nearest lower figure to the 4.7, or 4.5 in this case. Reading across the table from 4.5 to the column headed 70, it will be found that 7.38 pounds of low-lac should be added. The figures for additional sugar and M.S.N.F. are located in the same way.

It will be observed that the more efficient is the separation of lactose from the condensed skim milk, the smaller will be the quantity of low-lac required in the mix. It should also be pointed out that when the sum of the M.S.N.F. added to a mix in the low-lac and in the cream, exceeds the total desired percentage of M.S.N.F., then the upper limit of M.S.N.F. percentage in a given mix has been reached for a low-lac with this particular percentage of lactose removed.

MANUFACTURE OF ICE CREAM FROM LOW-LAC MILK

Ice cream mixes of varying composition were made up using low-lac to increase the milk solids above 10 per cent. Thirty-pound mixes were pasteurized for 30 minutes at 63° C., aged overnight, frozen, hardened and judged by from 10 to 15 persons within a week after freezing. A summary of the data obtained is given in table 4. The numerical scores have been summarized and the results reproduced in the table. Mix No. 1 in each experiment is the control mix for that experiment. It will be observed that in experiments No. 2 and No. 3 low-lac powder was substituted for the low-lac condensed milk. This was an entirely satisfactory spray dried product. However, the control mix in experiment No. 2 was preferred over those which contained low-lac powder. Again in experiment No. 7 the control ice cream was judged preferable over the low-lac product. However, since this latter control contained 12 per cent serum solids, it soon became sandy after slight heat-shocking.

The data in table 4 show that low-lac can be advantageously used to raise the milk-solids-not-fat of ice cream mixes from 10 to 12 per cent. Such ice creams generally show an improved body and texture, a higher total score, and are free from any susceptibility to sandiness. The flavor of the control samples was generally considered equal to or superior to the ice creams containing low-lac. However, the low-lac mixes possessed a firmer, smoother body and texture.

The removal of much of the lactose from skim milk and the subsequent use of this milk in the mix increases the ratio of milk salts to the other milk solids in the product. The effect of this altered ratio is to limit the quantity of milk-solids-not-fat which a low-lac mix may contain to about 13 per cent. This means that if a mix of 13 per cent serum solids is made from a batch of low-lac testing 62.5 per cent total solids and from which 67 per cent lactose has been removed, 6 per cent of the 13 per cent milk-solids-not-fat will be derived from the low-lac and the remaining 7 per cent from

normal sources of supply such as skim milk or cream. It is interesting to note that the proportion of serum solids to be derived from low-lac, when a good separation of lactose has been obtained, is less than half of the total amount in the mix and very often it will not exceed a quarter of the milk-solids-not-fat of the mix.

The low-lac provided a means of successfully substituting milk-solids-not-fat for fat to a limited extent as shown by the data in table 4, experiment 5. The ice cream containing 10 per cent fat and 12 per cent milk-solids-not-fat received a higher score than that containing 12 per cent fat and 10 per cent milk-solids-not-fat.

SUMMARY AND CONCLUSIONS

1. Approximately 65 per cent of the lactose of skim milk has been removed both on a laboratory and on a semi-commercial scale without altering the normal degree of dispersion of the milk proteins.

2. This process consists of the following steps: 5.9 pounds of cane sugar is added to 100 pounds of fresh skim milk, the mixture is forewarmed to 63° C. for 10 minutes, evaporated under vacuum to 70 per cent total solids, drawn and cooled to 25° C., held 20 hours at 10° C., and the crystallized lactose removed from the milk by means of a centrifuge or a filter press.

3. The value of low-lactose milk lies in the opportunity which it provides of increasing the milk-solids-not-fat of an ice cream mix above that amount where lactose crystallization threatens when normal milk solids are used to reach an identical concentration. Ice cream mixes containing 11 to 13 per cent milk-solids-not-fat, in which the concentration of lactose was controlled by the use of low-lac milk, possessed an improved body and texture and were capable of withstanding adverse handling conditions without the development of sandiness.

ACKNOWLEDGMENT

The authors express their appreciation and thanks to W. T. Johnson, Jr., and G. A. Ramsdell for their cooperation and help in applying on a semi-commercial scale, at the Grove City Creamery, this method of lactose separation, and to E. O. Whittier for checking the calculations introduced in this paper.

REFERENCES

- (1) GRAY, E. C., and TURNBOW, G. D. Frozen food product and method of making same. U. S. Patent 1,878,127, Sept. 20, 1932.
- (2) LEIGHTON, ALAN, and LEVITON, ABRAHAM. The effect of the diluting action of cane sugar upon the viscosity of the colloidal suspension skim milk. *Jour. Phys. Chem.* **36**: 523-528 (1932).
- (3) ASSOCIATES OF ROGERS. Fundamentals of Dairy Science, p. 33 (1928). The Chemical Catalog Co., Inc., New York, N. Y.

FURTHER STUDIES OF THE EFFECT OF PASTEURIZATION ON THE BACTERIAL FLORA OF LOW COUNT MILK

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The absence of acid coagulation and the development of abnormal flavors have been noted frequently in commercially pasteurized milk from different sections of the State of Washington. Similar observations have been made in other states. A recent correspondent (5) of a dairy trade journal made the following statement, which is typical of comments concerning this problem: "I have come to the conclusion that pasteurization equipment is becoming too efficient these days. By this I mean that too many of the lactic acid organisms are being killed and in this condition other organisms thrive which otherwise would have been retarded if a normal number of the lactic type had been left."

Data covering all seasons of the year and including more than 30,000 plate counts compiled from the records of numerous state, county, city, and milk plant laboratories located in various sections of the State of Washington show that approximately 35, 55, and 80 per cent of the market milk of the state reaches the pasteurizing plant with bacterial counts of less than 5,000; 10,000, and 25,000 per cc. respectively.

A study made by Black *et al.* (4) in this laboratory showed that when milk of low count was pasteurized in flasks at 142.5° F. for 30 minutes the proportion of acid-forming bacteria was reduced and that they were not of major importance in the spoilage of the product. Since the above study was made with samples of milk pasteurized in flasks and handled in a manner to prevent subsequent contamination, it seemed advisable to continue this study with the commercially pasteurized product.

LITERATURE REVIEW

Ayers and Johnson (2), from a study of the changes developed in litmus milk by organisms picked from plate cultures, found that pasteurization at 145° F. for 30 minutes caused a marked increase in the relative proportion of acid-forming bacteria to the other types in the milk. They reported similar findings when pasteurization was carried out at both 160° F. and 170° F., but at 180° F. the peptonizing bacteria predominated in the finished product. These findings were obtained on milk of a high initial count, many of the samples containing millions of bacteria per cc.

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The data presented by Ayres and Johnson (2) have been cited in a recent revision of a bulletin originally prepared by Ayers (1) in which it is stated that pasteurized milk sours in a manner similar to that of a high grade raw milk, except that the souring is delayed.

Recently Thurston and Olson (6), using the milk tube technique of Ayers and Johnson (2), found that pasteurization at 145° F. for 30 minutes reduced the percentage of acid-coagulating bacteria and increased the percentage of acid-forming non-coagulating types. The total percentages of these two groups remained unchanged, being 66.18 and 66.86 respectively in the raw and pasteurized milk. They studied the changes in the bacterial flora of six raw and six pasteurized samples held at storage temperatures ranging from 35° to 56° F. The acidities in both the raw and pasteurized samples increased but little during the four day observation period. The pasteurized milks stored at 53° to 56° F. were the only pasteurized samples the acid-forming, the proteolytic, and a group including alkali-forming and in which an appreciable increase occurred in the numbers of organisms. The predominating organisms in the pasteurized samples stored at this temperature after the first day were the inert and alkali-forming types. They found that acid developed slowly in the pasteurized milk and that coagulation occurred at low acidities apparently due to the production of a rennin-like enzyme by the organisms.

PROCEDURE

The data to be presented were obtained from 75 corresponding samples each of raw and pasteurized milk collected during all seasons. All samples represented the mixed milk from several herds. The raw milk samples were collected after the contents of the pasteurizer had been thoroughly mixed and before any temperature increase. Pint bottles of milk from the regular run constituted the pasteurized samples.

Pasteurization was carried out in a Burrell Simplex Spray Pasteurizer, of 200 gallon capacity, at 143° F. to 145° F. for 30 minutes and was followed by immediate cooling and bottling. Steam and hot water, of which there was an abundance, were used in the sterilization of the milk plant equipment.

The bacteria contained within the milk were divided into three groups: inert types. Total and differential bacterial counts were made on the raw and pasteurized samples when fresh and again after 24 hours and 48 hours of incubation at 70° F. This temperature was chosen for the reasons that it is favorable for the development of lactic-acid-forming bacteria and that bottled milk often is subjected to relatively high temperatures for considerable periods before being consumed.

The bacterial counts were determined by the use of Bacto-nutritive caseinate agar, a modification of the medium recommended by Ayers and Mudge

(3). Prior to sterilization 8 cc. of brom cresol purple indicator, prepared by dissolving 0.4 grams of the powdered indicator in 7.4 cc. of N/10 NaOH and 92.6 cc. of distilled water, were added to each liter of medium. Acid-forming colonies on this medium appear yellow. After counting the acid-forming colonies, the proteolytic types were differentiated by flooding the plate with dilute acetic acid. These colonies are recognized by the clear halos surrounding them. The alkali-forming and inert group was enumerated by subtracting the sum of the acid-forming and proteolytic types from the total number of colonies. Plate cultures were incubated at 20° C. for 96 hours.

Titrateable acidity was determined as percentage of lactic acid.

EXPERIMENTAL DATA

The average numbers of bacteria per cc., the average percentage of each bacterial group, and the average acidities in fresh milk and in the milk incubated at 70° F. for 24 and 48 hours are shown in table 1.

TABLE 1

Average numbers of bacteria per cc., average percentages of each bacterial group and average acidities in 75 corresponding samples of fresh raw and pasteurized milk and in raw and pasteurized milk incubated at 70° F.

TYPE OF MILK	BACTERIA PER CC.	PER CENT ACID FORM- ING	PER CENT PROTEO- LYTIC	PER CENT ALKALI- FORMING & INERT	PER CENT ACID	PER CENT ACID COAGU- LATION
Fresh raw milk	14,800	29.40	7.00	63.60	0.16	—
Fresh pasteurized milk	2,070	18.80	5.00	76.20	0.16	—
Raw milk incubated 24 hours	241,000,000	92.10	0.55	7.35	0.26	0.0
Pasteurized milk in- cubated 24 hours	7,300,000	12.40	1.20	86.40	0.17	0.0
Raw milk incubated 48 hours	1,500,000,000	96.00	0.04	3.96	0.79	98.7
Pasteurized milk in- cubated 48 hours	960,000,000	76.10	0.20	23.70	0.55	57.3

DISCUSSION OF RESULTS

The initial bacterial counts of the 75 raw milk samples ranged from 2,400 to 50,000 per cc. Forty-four per cent contained less than 10,000 per cc. and 82.7 per cent gave counts of less than 25,000 per cc. The raw samples had an average count per cc. of 14,800 as compared to 2,070 for the freshly pasteurized samples.

Pasteurization resulted in an average reduction in bacterial numbers of 86 per cent, a decrease in the percentage of acid-forming bacteria from

29.4 to 18.8, a decrease in the percentage of the proteolytic group from 7.0 to 5.0 and an increase from 63.6 to 76.3 per cent in the alkali-forming and inert group.

After 24 hours of incubation the percentage of acid-forming bacteria in the raw and pasteurized samples of milk was 92.1 and 12.4, respectively. No significant difference occurred in the proportion of the proteolytic group in the two kinds of milk. The alkali-forming and inert group constituted 7.35 per cent of the raw milk flora as compared to 86.4 per cent of the flora of the pasteurized milk. Acid had not increased in any sample sufficiently to bring about coagulation. The flavors of the pasteurized samples, in all instances, were equal if not superior to the flavors of the corresponding raw samples. Many of the raw samples after 24 hours had developed sufficient acid to cause an undesirable flavor.

After 48 hours results were obtained that varied considerably from those secured after 24 hours. Less difference occurred in the percentages of the acid-forming and the alkali-forming and inert groups of the raw and pasteurized milk. The acid-forming bacteria constituted 96 per cent of the raw milk flora as compared to 76.1 per cent of the pasteurized milk flora. The alkali-forming and inert types of bacteria composed 3.96 and 23.7 per cent, respectively of the flora of the raw and pasteurized samples. Of the raw milk samples 98.7 per cent showed acid coagulation as compared to 57.3 per cent of the pasteurized samples. In 20 per cent of the pasteurized samples less than 0.4 per cent acid was present at the end of 48 hours. A pronounced bitter flavor had developed in all of the low acid samples and in a number of instances sweet curdling had taken place.

A period of delayed activity of the acid-forming bacteria followed by a period of increased activity was noted in the pasteurized samples. The proportion of these organisms increased from 12.4 to 76.1 per cent from the 24th to the 48th hour. In this same period a decrease occurred in the proportion of the alkali-forming and inert group. During the period in which the acid-producing organisms were relatively inactive there was an increased activity of the alkali-forming and inert group, probably accounting for the bitter flavor occurring in the samples of low acid content. Of the samples having less than 0.4 per cent acid at the end of 48 hours the alkali-forming and inert group of bacteria was in the majority, composing 52.4 per cent of the flora as compared to 46.7 per cent for the acid-forming organisms. After 72 hours the acid content of all these samples had increased to 0.6 per cent or more.

The results of this study show a considerable variation in details from those secured by Black *et al.* (4). This is to be expected since they were dealing with milk pasteurized in flasks and handled in such a way as to prevent subsequent contamination. However, the results support the findings of the above investigators as well as those of Thurston and Olson (6)

and furnish additional evidence that the spoilage of low count commercially pasteurized milk, in a great many instances, is due to the action of organisms other than the lactic acid producing types.

When pasteurized milk is consumed within 24 hours after delivery there is usually little danger of abnormal fermentations, such as encountered in this study, being noticeably present. When the product is kept an additional 12 to 24 hours at room temperature, as is often the case, pronounced abnormalities other than souring may be in evidence. If it is desired to overcome this condition, pure cultures of lactic acid producing organisms might be added to the pasteurized product prior to the bottling operation. Such a practice, however, would make it necessary to revise our present bacterial standards as the score of milk is determined largely by the number of organisms rather than by the types that are present.

SUMMARY AND CONCLUSIONS

Total and differential bacterial counts were made on 75 samples each of raw and commercially pasteurized milk while fresh and again after 24 and 48 hours of incubation at 70° F.

Pasteurization resulted in a decrease in the percentage of the acid-forming bacteria from 29.4 to 18.8, a decrease in the percentage of the proteolytic group from 7.0 to 5.0 and an increase from 63.6 to 76.2 per cent in the alkali-forming and inert group.

After 24 hours the percentage of acid-forming bacteria in the raw and pasteurized samples was 92.1 and 12.4 respectively. No significant differences existed in the proportion of the proteolytic group in the two kinds of milk. The alkali-forming and inert group constituted 7.35 per cent of the raw milk flora as compared to 86.4 per cent of the flora of the pasteurized samples.

After 24 hours the flavors of the pasteurized samples were more desirable than the flavors of the raw samples.

After 48 hours 98.7 per cent of the raw milk samples showed acid coagulation as compared to 57.3 per cent of the pasteurized samples. Ninety-six per cent of the raw milk flora were acid-forming organisms as compared to 76.1 per cent of the pasteurized milk flora. The proteolytic group constituted less than 1.0 per cent of the flora of either type of milk. The alkali-forming and inert group made up 3.96 per cent of the flora of the raw milk and 23.7 per cent of the flora of the pasteurized milk. Twenty per cent of the pasteurized samples developed a pronounced bitter flavor without the accumulation of more than 0.4 per cent acid.

The results obtained in this study bear out the contention that, in many instances, organisms other than the lactic acid types are of major importance in the spoilage of commercially pasteurized milk derived from low count raw milk.

REFERENCES

- (1) AYERS, S. HENRY. The present status of the pasteurization of milk. Revised by R. P. Hotis and C. J. Babcock. U.S.D.A. Bul. No. 342, issued Jan. 1916; revised June 1932.
- (2) AYERS, S. HENRY, AND JOHNSON, WILLIAM T. A study of the bacteria which survive pasteurization. U.S.D.A. Bur. of Animal Ind. Bul. 161. 1913
- (3) AYERS, S. HENRY, AND MUDGE, COURTLAND S. Milk powder agar for the determination of bacteria in milk. Jour. of Bact. 5: 565. 1920.
- (4) BLACK, L. A., PROUTY, C. C., AND GRAHAM, R. A. The effect of pasteurization on the bacterial flora of low count milk. JOURNAL OF DAIRY SCIENCE 15: 99. 1932.
- (5) Ropy milk and lactic bacteria. Milk Plant Monthly 22: No. 6, 56, June 1933.
- (6) THURSTON, L. M., AND OLSON, H. C. The bacterial flora of high-grade milk before and after pasteurization. West Virginia Agr. Expt. Sta. Bul. 225, June 1933.

American Dairy Science Association Announcements

ANNUAL MEETING AMERICAN DAIRY SCIENCE ASSOCIATION

Ithaca and Geneva, New York

June 26 to 28, 1934

The first two days of the meeting will be held at Ithaca, and the third day (June 28) will be at Geneva. There will be conferences and demonstrations on judging dairy cattle and products in Ithaca on June 25, the day preceding the regular program. Rooms at a moderate rate will be furnished at Ithaca for the nights of June 25, 26, and 27. Further details will be published in the subsequent numbers of the JOURNAL OF DAIRY SCIENCE.

SCIENTIFIC PROGRAM

Members are invited to send titles of papers to the program committee. Papers will be limited in length to fifteen minutes. With the exception of invited speakers, at least one author of each paper must be a member of the American Dairy Science Association. Papers should represent original work not previously published.

The members of the program committee are Fordyce Ely, H. A. Ruehe, and J. M. Sherman, chairman. *Titles of papers must be received by the chairman of the program committee (Dairy Industry Building, Ithaca, N. Y.) before May 1.*

Members are asked to accept this invitation to send in papers as the official notice of the meeting, as individual notices will not be sent to members from the secretary's office.

NEWS ITEM

J. E. Dorman retired from the Bureau of Dairy Industry, U. S. Department of Agriculture at the close of the calendar year 1933 after more than 30 years in the Government service. His first connection with the Bureau was extension work in the South. Later he helped in the selection of the Beltsville Dairy Farm, Beltsville Md., and superintended the construction of its first buildings. Mr. Dorman was in charge of the Extension Service office of the Bureau of Dairy Industry, with headquarters in Salt Lake City, from 1913 to 1929. During the past 5 years he has been in charge of the summarization of records in the dairy herd-improvement association work with headquarters in Washington, D. C.

Mr. Dorman was one of the best known men in the Bureau, and one of the most respected men in the dairy industry. At the close of his last day in the Service, the Bureau held a reception in his honor at which Chief O. E. Reed gave an outline of the splendid service the Bureau had received from Mr. Dorman. Mr. Dorman responded in his characteristically humorous style.

Mr. Dorman expects to live in Florida. His fellow workers and friends wish him a long and happy life.

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THE EFFECT OF SPECIFIC DIETARY FATS ON THE BLOOD LIPIDS OF LACTATING GOATS¹

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Several studies (1, 2, 3, 4, 5) dealing with the relationship of the various blood lipids in lactating animals to the fat intake on the one hand, and to the milk and fat secreted on the other, have been reported from this laboratory. These studies have added considerably to our knowledge of the physiology of lactation, and made it evident that a consideration of these blood lipid relationships is of distinct value for obtaining a more exact understanding of the dietary requirements for maximum milk and fat production.

In our studies with cows dealing with the influence of different fat intakes upon the blood picture and upon production, the dietary modifications were limited to the partial or nearly complete extraction of the fat from the grain mixture and its replacement by starch. The results obtained suggested the desirability of ascertaining the effect of a more complete removal of the fat from the ration and the influence of specific kinds of fat. Since such studies with cows would prove very expensive, and impracticable in other respects, we were led to employ goats for the purpose. One report of this work has already appeared in this Journal (4). The present article describes further experiments involving a study of the amount and distribution of the various lipids in the plasma and cells, as influenced by specific oils and fatty acids, when substituted for starch in a fat-free ration.

EXPERIMENTAL PROCEDURE

In order to obtain a basal diet as free from fat as possible an extracted grain mixture was used as in the previous studies and regenerated cellulose was used as the roughage component in place of hay. This cellulose was a purified product practically free from ether extractible material. The grain mixture was made up as follows:

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¹ The data presented are taken from a thesis offered by H. H. Williams to the Graduate School of Cornell University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

20 parts	Hominy feed
20 parts	Ground oats
25 parts	Wheat bran
22 parts	Corn gluten feed
10 parts	Corn gluten meal
1 part	Bonemeal
1 part	Ground limestone
1 part	Salt

By extracting this grain mixture with 95% alcohol continuously for 3 days in an extractor designed by McCay (6), its ether extract was reduced to less than 0.2%. The fat thus removed was replaced by an isodynamic amount of starch. In addition to the grain mixture and cellulose the basal diet contained molasses, ether-extracted yeast, White's vitamin A-D concentrate and additional minerals. The molasses was used primarily for its effect on palatability. The yeast and A-D concentrate were added to supply vitamins. The minerals consisted of equal parts of bonemeal, limestone and salt plus 1% of ferrous sulfate and 0.1% of potassium iodide.

The oils and fatty acids employed as supplements to the basal diet were butter oil, coconut oil, a mixture of palmitic and stearic acids, and a mixture of oleic and linoleic acids. The butter oil represented the same fat as secreted in the milk. The coconut oil provided a nearly saturated fat having a fatty acid distribution somewhat similar to that of milk fat. The specific fatty acids used provided for a comparison of the effect of saturated fatty acids of high molecular weight (palmitic and stearic) with unsaturated acids of high molecular weight (oleic and linoleic).

Six goats, approximately one month along in their lactation, were used over an experimental period of 112 days, divided into four sub-periods of 28 days each, with the exception that the first sub-period was 31 days for four of the animals. During the first period each goat received the fat-free ration. During subsequent periods various diets involving the use of the fat supplements were employed as shown in table 2. It is noted in this table that in the case of four of the animals alfalfa hay was included in the ration during the final period. The reason for this is explained later.

Throughout the experiment, with the exception of the cases where alfalfa was fed, each animal received daily 200 grams of cellulose, 200 grams of molasses, 25 grams of extracted yeast, 20 grams of the mineral mixture, and 4 drops of A-D concentrate. The grain mixture, with or without fat additions, was varied from week to week according to the production of the animal during the previous week with the object of supplying digestible crude protein and total digestible nutrients in accordance with the animal's requirements. In general for a given animal the grain intake ranged between 400 and 600 grams daily. The entire basal diet as fed contained approximately 0.2% of fat. Where fat additions were made the amounts were governed by the amounts being secreted in the milk. Where alfalfa and

grain were fed these feeds were allowed *ad libitum*. The daily intakes of total digestible nutrients and of fat are shown in table 1. It is noted that the intakes of total digestible nutrients declined as the experiment advanced, with the exception of the last period where hay and grain was fed *ad libitum*. This is in part the result of a lower allowance due to the drop in milk yield and in part to frequent failures of the animals to eat their allowance completely. In the last periods for goats A, C, E, and F, it is probable that the *ad libitum* feeding of grain and hay resulted in an unnecessarily high intake of total digestible nutrients. The possibility that the animals were underfed when on the other diets will be referred to in discussing the milk yields.

TABLE 1

Daily intakes of total digestible nutrients and fat, averaged by periods

GOAT	PERIOD 1		PERIOD 2		PERIOD 3		PERIOD 4	
	T. D. N.	Fat	T. D. N.	Fat	T. D. N.	Fat	T. D. N.	Fat
	<i>gms.</i>	<i>gms.</i>	<i>gms.</i>	<i>gms.</i>	<i>gms.</i>	<i>gms.</i>	<i>gms.</i>	<i>gms.</i>
A	771	2.2	650	50.6	549	1.6	956	14.4
B	591	2.3	364	12.9	357	14.2	334	1.0
C	670	2.2	603	36.2	589	34.1	1052	49.9
D	706	2.2	501	22.1	444	19.2		
E	647	2.0	500	26.5	490	18.9	900	* 13.5
F	634	2.0	505	24.8	476	17.3	1003	47.9

It was originally planned to take blood samples at the beginning, in the middle, and at the end of each period. After the first period, however, it was found that there was insufficient time for such a large number of analyses, and thus thereafter the sampling was frequently limited to one taken at the end of each period.

The blood plasma and cells were analyzed for total and free cholesterol, lipid phosphorus, total lipids, and the iodine number of the total lipids. The cholesterol analyses were carried out by Okey's (7) procedure as modified by Yasuda (8). The lipid phosphorus of the alcohol-ether extract was determined by Denige's procedure as modified by McCay (9). This was multiplied by the factor 18.26 to get the amount of phospholipid fatty acids. The total lipids were determined by Bloor's oxidation procedure (10) and the iodine numbers of the total lipids were obtained by the method of Yasuda (11).

RESULTS

In our previous experiments with cows and goats, it has been shown that the partial or complete removal of the fat from the grain mixture causes a decline in milk yield. In planning the present experiment, it was hoped to

obtain some definite information as to the influence of specific fats upon milk and fat yield as well as on the blood picture. While these records for yield were obtained, the detailed data are not being reported at this time pending the completion of a further experiment which it is hoped will answer questions now obscuring the significance of the present results. However, a brief statement regarding these data will be made for reference in connection with the discussion of the blood data, and to indicate the questions requiring further study.

In every case the fat-free ration caused a very rapid drop in milk yield and an equal or greater decline in fat yield. These declines ranged from 25 to 65% for the various goats from the start to the end of the period. In no case did the inclusion of a fat supplement in the ration have any effect on milk yield beyond arresting wholly or in part the decline on the fat-free ration, although the butter oil and coconut oil increased the fat percentage. Because of these results for milk yield, which were contrary to what we had obtained with cows, the question arose as to whether any system of feeding would cause any marked increase in milk yield in goats after they had experienced such a severe decline as occurred on our basal diet. To answer this question a ration of alfalfa hay and grain was fed *ad libitum* to four of the goats (A, C, E, F) in the final period. In two cases the grain mixture had been extracted, in the other two, not. In all cases the milk and fat yields rose markedly. However, as previously mentioned in referring to table 1, the consumption of total digestible nutrients was considerably greater than in the previous periods. Thus the results with the hay and grain, which answered the specific question causing them to be fed, raised the further question as to the significance of the results during the previous periods. It is possible that the large drops in yield during the fat-free period and the failure of the fat additions to increase the yield were due to an insufficient intake of food, although the animals maintained their weights throughout these periods. The superior results with the alfalfa ration may have been due merely to a larger intake of food, or to the removal of some specific unfavorable factor in the previous diets, or to the addition of an unrecognized essential in the alfalfa. These are the questions now under study.

The blood data are presented in table 2. It is noted that in some periods the analyses were made three times in a given period, in others twice, and in the remainder once, with the exception that in period 3 no blood sample was taken from goat B. Where only one analysis was made during a period the sample was taken at the close in order to measure the final effect of the diet over the 28 days.

The data for total lipids in the plasma show that in every case they dropped markedly on the fat-free diet. In the case of goats A and B, where this diet was followed by the one containing the butter oil, the total lipids

TABLE 2
The distribution of total lipids, total and free cholesterol, and phospholipids in the plasma and cells—milligrams per 100 cc. The iodine numbers of the total lipids

GOAT NO.	PERIOD	DIET	DAYS ON DIET	TOTAL LIPIDS				CHOLESTEROL				PHOSPHOLIPID FATTY ACIDS	
				Plasma		Cells		Plasma		Cells		Plasma	Cells
				Amt.	I. N.	Amt.	I. N.	Total	Free	Total	Free		
A	1	Fat-free	5 19 31	325 300 261	73 66 65	560 553 444	60 64 54	76 65 51	15 17 11	154 140 142	146 131 134	82 73 60	250 217 248
	2	Fat-free and butter oil	2 25	327 482	67 68	479 503	64 53	77 129	17 30	145 155	124 147	82 137	219 267
	3	Fat-free	27	234	53	464	53	37	10	156	151	60	265
	4	Extracted grain & alfalfa	27	328	59	475	52	67	13	136	134	86	252
B	1	Fat-free	5 19	351 300	67 59	604 552	66 65	91 45	27 16	190	162	84 57	230 245
	2	Fat-free and butter oil	1 6 27	279 322 351	55 63 73	546 542 525	64 66 70	54 62 80	13 14 20	157 147 143	145 147 143	68 79 102	245 245 267
	3	Unextr. grain & butter oil											
	4	Fat-free	3 26	322 268	63 54	559 536	66 66	60 59	14 11	136 150	136 150	82 62	265 268
C	1	Fat-free	6 20 31	231 194 195	73 71 62	550 563 504	62 63 61	61 53 47	13 9 12	150 157 129	150 155 123	69 58 57	254 256 223
	2	Fat-free & stearic & palmitic acids	2 25	238 308	67 62	496 529	62 58	62 86	13 19	120 141	120 138	71 91	247 245
	3	Unextr. grain & stearic & palmitic acids											
	4	Unextr. grain & alfalfa	27	295 360	65 66	533 500	60 67	73 85	17 20	141 140	115 140	86 99	223 223

TABLE 2—(Continued)

GOAT NO.	PERIOD	DIET	DAYS ON DIET	TOTAL LIPIDS				CHOLESTEROL				PHOSPHOLIPID FATTY ACIDS	
				Plasma		Cells		Plasma		Cells		Plasma	Cells
				Amt.	I. N.	Amt.	I. N.	Total	Free	Total	Free		
D	1	Fat-free	2	374	76	492	63	101	23	129	120	108	225
	25		25	270	54	479	64	59	12	127	127	69	230
	3	Fat-free & stearic & palmitic acids	3	290	57	494	61	60	10	129	127	82	239
E	3	Unextr. grain & stearic & palmitic acids	27	333	58	512	59	78	16	128	127	99	237
	1	Fat-free	27	344	66	506	60	85	20	135	121	110	237
	2	Fat-free & coconut oil	7	282	72	553	60	76	16	143	137	84	236
F	2	Fat-free & coconut oil	21	258	67	543	54	66	14	149	131	75	223
	3	Fat-free & oleic & linoleic acids	31	200	67	448	55	56	19	138	124	70	237
	4	Extracted grain & alfalfa	2	272	67	496	61	67	13	126	110	80	217
F	3	Fat-free & oleic & linoleic acids	26	239	57	425	57	61	18	131	131	75	247
	4	Extracted grain & alfalfa	27	273	63	497	61	86	25	151	149	100	274
	1	Fat-free	27	222	67	444	56	63	14	123	117	75	239
F	2	Fat-free & coconut oil	7	285	62	523	60	43	14	147	138	66	214
	3	Fat-free & oleic & linoleic acids	21	248	54	517	55	36	10	133	124	57	212
	4	Unextr. grain & alfalfa	31	231	58	448	49	31	10	129	127	55	221
F	2	Fat-free & coconut oil	2	195	57	499	60	42	10	127	123	55	214
	3	Fat-free & oleic & linoleic acids	26	299	65	458	51	49	11	134	129	71	230
	4	Unextr. grain & alfalfa	27	276	58	500	51	55	12	144	136	73	241
F	2	Fat-free & coconut oil	27	330	62	444	54	62	17	133	132	82	228

rose on the latter, to fall again when the fat-free ration was restored. The diet of extracted grain and alfalfa hay fed to goat A in the final period also caused a rise. The stearic and palmitic acids, fed to goats C and D along with either extracted or unextracted grain, produced the same results as the butter oil in causing the plasma lipids to rise, following the drop on the fat-free ration. Similarly, as shown by the data for goats E and F, these lipids were higher during the period when coconut oil was fed, and also when the mixture of linoleic and oleic was given, than at the close of the period on the basal diet. Higher values are also shown where hay and grain were fed to goats C, E, and F. A comparison of all of these data for plasma total lipids in table 2, with those for fat intake in table 1, shows that the lipid values were at their lowest on the fat-free ration and uniformly higher when fat was added either as an oil, a fatty acid or in a natural feed. It is also noted in those cases where more than one determination was made in a period that in general the response to a change in fat intake was a gradual one. This is in accord with the observations in this laboratory and elsewhere for other herbivorous animals.

The figures for cell total lipids show in general a decrease on the fat-free diet but to a much smaller degree than in the plasma. The changes occurring with the fat additions are small and show no certain trend. Clearly the character of the diet exerts its influence primarily on the lipids in the plasma rather than in the cells.

The data for the iodine numbers of the total lipids in the plasma show that these values gradually fell on the fat-free diet. This was to be expected in view of the previous observations (1, 4) with both cows and goats that a low-fat diet causes the iodine numbers of the milk fat to drop and is in accord with the recent results obtained by Hansen and Burr (12) for the rat. While the data are limited and not entirely in agreement, the changes resulting with the use of the various diets containing fat are mostly in the direction expected from the iodine number of the fat fed. An extensive study is now being made in this laboratory of the relation of the iodine numbers of the various plasma lipids to the character of the food on the one hand, and the character of the milk fat on the other. As is borne out by the data here reported, it has been found that dietary fats of widely differing iodine numbers, which produce immediate and marked differences in the iodine numbers of the milk fat, cause only small differences in the total lipids in the plasma. This means that a large number of observations must be made to obtain significant results. It is believed, however, that the data which are being accumulated will contribute to our knowledge of the source of milk fat and of the physiological changes involved.

In contrast to the data obtained for the plasma, the iodine numbers of the cell lipids, though somewhat variable, fail to reveal any changes which can be correlated with the nature of the diet. Thus it is seen that, as re-

guards state of saturation as well as amount of total lipids, the changes in the diet are reflected primarily in corresponding changes in the plasma and not in the cells.

The data for phospholipid fatty acids in the plasma show that they decreased during the fat-free period and rose with the addition of fat, thus paralleling the changes noted for the total lipids of which they made up a part. The phospholipid fatty acids are shown to occur in much larger amounts in the cells than in the plasma, as was also true, though to a lesser degree, for the total lipids. However, the changes which are shown for these acids in the cells are slight and bear no relation to the fat content of the diet, a finding which is not unexpected in view of the data for total lipids in the cells.

The data for cholesterol are of special interest in view of the mystery that surrounds the physiological significance of this compound. In the plasma the total cholesterol is shown to decrease on the fat-free diet and to rise with the addition of fat regardless of type. This finding is in accord with those for other species that the total cholesterol in the plasma tends to parallel the level of fat intake. It is noted that only a small part of this cholesterol is in the free state but that its level tends to parallel that of the total. The fact that most of the cholesterol of the plasma is combined with fatty acids and that the amount varies with the fat intake furnishes evidence for the view that cholesterol plays an active rôle in fat metabolism in lactation.

In the cells a very different picture is shown. Here the total cholesterol greatly exceeds that in the plasma. It is almost entirely present in the free state in contrast to the situation in the plasma where the major portion is present as ester. Further, this cell cholesterol remains fairly constant in amount and such changes as occur bear no relation to the changes in diet.

In general, the blood changes shown in table 2 present a picture similar in kind to that reported for non-lactating animals of other species. It thus appears that the constant withdrawal of blood lipids for the secretion of milk does not alter the essential nature of the changes in these lipids which are brought about by changes in the diet. In the previous experiments with cows (1, 3) and to a lesser degree with goats (4) the addition of fat to a low-fat diet had a positive effect upon milk and fat yield as well as upon the lipid level of the blood, suggesting the conclusion that the blood lipid level is a factor in milk yield. The very limited effect thus obtained in this experiment, except with the hay and grain, has been referred to and the possible explanations now under study have been cited. A fat-free diet which is more satisfactory as regards palatability, and perhaps also in other respects, must be devised before the full significance of results such as have been obtained in the present experiment can be determined. While the omission from the diet of ruminants of their natural roughage and the sub-

stitution of purified nutrients have obvious disadvantages, the successful use of such a controlled diet, which enables the experimenter to know the exact nature of the nutrients he is feeding and omit any one at will, should give a much more exact picture of the metabolism in milk producing animals than we have at present.

SUMMARY

The total lipids, phospholipids, free and combined cholesterol and the iodine numbers of the total lipids have been determined in the plasma and cells of the blood of lactating goats receiving in one period a nearly fat-free ration and in other periods the same ration plus certain oils and fatty acids. The fat-free diet consisted of an extracted grain mixture, regenerated cellulose, starch, molasses, extracted yeast, an A-D concentrate and a mineral mixture. The oils and acids used were butter oil, coconut oil, palmitic and stearic acids, and oleic and linoleic acids.

In the plasma the total lipids, phospholipids and total and free cholesterol dropped gradually on the fat-free diet and rose on the diets containing the fat additions irrespective of the nature of the fat fed. The iodine numbers of the total lipids in the plasma fell on the fat-free diet. With the inclusion of fat in the diet these numbers tended to change in the direction expected on the basis of the iodine number of the fat fed. In the cells the various lipid values tended to remain constant and showed no changes which could be correlated either with the nature of the diet or the changes in the plasma. The cholesterol in the cells was almost entirely in the free state while in the plasma it was present chiefly in the combined form.

In general this blood picture is similar to that reported for non-lactating animals of other species. It thus appears that the constant withdrawal of blood lipids for the secretion of milk does not alter the essential nature of the changes in these lipids which are brought about by changes in the diet.

REFERENCES

- (1) MAYNARD, L. A., AND McCAY, C. M. The influence of a low-fat diet upon fat metabolism during lactation. *Jour. Nutrition* 2: 67. 1929.
- (2) MAYNARD, L. A., HARRISON, E. S., AND McCAY, C. M. The changes in the total fatty acids, phospholipid fatty acids, and cholesterol of the blood during the lactation cycle. *Jour. Biol. Chem.* 92: 263. 1931.
- (3) MAYNARD, L. A., AND McCAY, C. M. The influence of different levels of fat intake upon milk secretion. *Cornell Agr. Expt. Sta. Bul.* 543. September, 1932.
- (4) BENDER, R. C., AND MAYNARD, L. A. Fat metabolism in the lactating goat. *JOUR. DAIRY SCI.* 15: 242. 1932.
- (5) SCHABLE, P. J. Plasma lipids in lactating and non-lactating animals. *Jour. Biol. Chem.* 95: 79. 1932.
- (6) McCAY, C. M. A continuous extractor of large capacity. *Ind. & Eng. Chem., Anal. Ed.* 5: 213. 1933.

- (7) OKFY, R. A micro method for the estimation of cholesterol by oxidation of the digitonide. *Jour. Biol. Chem.* **88**: 367. 1930.
- (8) YASUDA, M. Contributions to the microdetermination of cholesterol. *Jour. Biol. Chem.* **92**: 303. 1931.
- (9) MCCAY, C. M. Phosphorus distribution, sugar, and hemoglobin in the blood of fish, eels and turtles. *Jour. Biol. Chem.* **90**: 497. 1931.
- (10) BLOOR, W. R. The determination of small amounts of lipid in blood plasma. *Jour. Biol. Chem.* **77**: 53. 1928.
- (11) YASUDA, M. The determination of the iodine number of lipids. *Jour. Biol. Chem.* **94**: 401. 1931.
- (12) HANSEN, A. E., AND BURR, G. O. Iodine numbers of serum lipids in rats fed on fat-free diets. *Proc. Soc. Exp. Biol. & Med.* **30**: 1201. 1933.

BY-LAWS OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

ARTICLE I—MEMBERSHIP

Section 1—Membership in this Association shall consist of two kinds, active and associate.

Section 2—Any person is eligible to active membership who is formally announced by an Agricultural College or Experiment Station, or by the Bureau of Dairy Industry of the United States Department of Agriculture, or by the Canadian Department of Agriculture as an instructor, extension worker, investigator, or administrative officer connected with the dairy industry, or any person filling a position of responsibility connected with the dairy industry who has had a college or university training in technical science, or any person filling a responsible position in the dairy industry of a professional character requiring a technical knowledge of dairying of a high order.

Section 3—Any person is eligible to associate membership who is regularly enrolled in a collegiate course in a college of agriculture and who is specializing in dairying.

Section 4—Nominations for active membership shall be submitted to the Secretary-Treasurer in writing signed by the applicant and endorsed by at least one active member. In case of uncertainty regarding the eligibility of the applicant for membership the Secretary-Treasurer shall refer the application to the Board of Directors for decision. Upon receiving the approval of the Secretary-Treasurer, or the Board of Directors when the application has been referred to them for action, and the payment of membership dues, the applicant shall be enrolled as an active member of the Association.

Section 5—Associate membership of this association shall consist of those persons elected to membership in a local chapter of the American Dairy Science Association. Election to such chapter shall be governed by the rules and regulations described by the local chapter, subject only to the qualifications for associate membership as hereinbefore specified, and the approval of the head of the Department of Dairying or Dairy Husbandry in the institution where the chapter is located. Associate membership expires two years after the holder ceases to be enrolled as a student.

Section 6—The active membership dues shall be \$5 a year, payable January 1st.

Section 7—Associate membership dues shall be \$0.50 a year, and shall be paid annually to such officer of the chapter to which such associate mem-

ber belongs as may be provided by the chapter. Such dues shall be forwarded by the chapter to the Secretary-Treasurer of the Association.

Section 8—Any member of the Association in arrears for dues for more than one year shall cease to be a member of the Association but may be restored without the formality of reelection by payment of current dues.

ARTICLE II—OFFICERS

Section 1—The officers of the Association shall be President, Vice-President, Secretary-Treasurer, Journal Editor, and a Board of Directors.

The Vice-President shall be elected by the vote of the active membership and his term of office shall be one year beginning October 1st following his election. On the completion of his term of office as Vice-President he shall automatically become President for one year or until his successor is duly chosen. The Secretary-Treasurer and the Journal Editor shall be elected by the Board of Directors for such term of office as the Board of Directors shall prescribe.

Section 2—The Board of Directors shall consist of three members elected by the active membership, the President, Vice-President, and Secretary-Treasurer, who shall be an ex-officio member.

At the first election under this constitution as amended, one Director shall be elected for a term of one year, one for a two-year term, and a third for a three-year term; thereafter one Director shall be elected each year, whose term of office shall be three years. The terms of all Directors begin October 1st.

Section 3—The Board of Directors shall elect two members from the Association, who with the Secretary-Treasurer as ex-officio member shall constitute the Journal Management Committee, which shall be responsible to the Board of Directors. The term of service of the elected members shall be at the discretion of the Board of Directors.

Section 4—The Board of Directors may constitute and appoint such committees not provided for in the By-Laws of the Association, as they may deem proper, from their own membership or from among the active membership of the Association.

Section 5—The Board of Directors shall have the authority to fill vacancies that may occur among the officers of the Association, such appointees to serve during the remainder of the unexpired term of the office in question.

ARTICLE III—DUTIES OF OFFICERS

Section 1—The President of the Association shall preside at all meetings of the Association and meetings of the Board of Directors and shall perform such other duties as pertain to that office. The Vice-President shall perform the duties of the President in the absence of the President.

Section 2—The Secretary-Treasurer shall have custody of the books and

records of the Association, keep the minutes of all meetings of the Association and of the Board of Directors, maintain a list of members, keep the funds of the Association and make disbursements therefrom when properly authorized.

Section 3—The Journal Management Committee shall have the general supervision of the Journal. The Journal Editor, under the general supervision of this Committee, shall have direct charge of the details of the editorial and business management.

Section 4—The Board of Directors shall pass upon all applications for divisions, sections, and chapters of the Association.

Section 5—The Board of Directors shall have full control of the business of the corporation, and the title to all property and funds of the Association shall be vested in the Board of Directors. The Board of Directors shall have all the rights and powers vested in the Corporation by the laws of the District of Columbia.

ARTICLE IV—ELECTION OF OFFICERS

Section 1—On or before August first, the Secretary shall mail to each active member a blank on which he shall be entitled to express his choice for each office to be filled. This blank shall give the names of those serving in the offices to be filled for five years preceding and a report of the Committee on Elections which shall suggest the names of two members for each office to be filled. All ballots shall be counted by the Secretary and later verified by the President. In case no candidate has a majority by the first ballot, a second ballot shall be sent to the members, giving the names of the three leading candidates for each office and the number of votes received. The candidates receiving the most votes on the second ballot shall be declared elected. In case of tie on the second ballot, the decision shall be made by the Board of Directors.

ARTICLE V—MEETINGS

Section 1—Meetings of the Association shall be held at the time and place fixed by the Board of Directors but not less than one each calendar year shall be held. Notice of the time and place of meetings of the Association shall be given to all active members not less than four weeks prior to the date of meeting.

Section 2—Meetings of the Board of Directors shall be held upon call of the President, provided, however, that not less than 10 days' notice of such meeting shall be sent to each member of the Board of Directors, and provided further, that the Board of Directors shall hold a meeting for the purpose of organization within one month after the annual election.

WATER REQUIREMENTS OF DAIRY CALVES *

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Calves subsist mainly on a fluid diet during the first few months of their lives. Many hand-fed calves are not offered water until they are several months of age. To obtain information on the amount of water consumed by calves, eight groups of Holstein calves under various feed conditions were observed. Both free water drunk and water consumed in feeds were recorded by weeks to obtain total water intake.

Writers on the subject of calf feeding universally stress the importance of an ample supply of fresh water, but a relatively small amount of data has been published on water consumption by calves. Henry and Morrison (4) state that "calves two to four months old, fed skimmilk, will consume from 10 pounds or less of water daily up to 20 pounds or more." Otis (7) found that at three months of age a calf will drink on the average five quarts of water daily. McCandlish (5) reported that calves drank 4 to 8 pounds of water per head daily in winter. Bowling and Ackerman (2) obtained slightly better gains in body weight and in height at withers with calves receiving no free water up to eight months of age than with calves receiving free water at will. Several investigators (1, 3, 6) have suggested that with older cattle the total water intake depended on the quantity of dry matter consumed. No report was found on the relative amounts of free water and total water consumed by calves at various ages while being fed milk.

PLAN OF EXPERIMENT

In this study water consumption by 30 Holstein-Friesian calves (excepting one Jersey-Holstein cross-bred) was recorded in connection with feeding trials. The calves were divided into groups of four each, excepting Group IV which represented only two calves. All groups were started between the months of December and March and continued for 180 days. Thus, the atmospheric temperature was relatively low when the data for early ages were taken and increased as the calves grew older.

Calves were housed in individual stalls so prepared that all feeds refused could be accounted for. Water was supplied in 14-quart pails. Check records showed evaporation to be a negligible factor. Records of consumption were summarized by weeks. Feeds were weighed and analyzed in connection with the feeding trials so that complete information was available. Hay was

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supplied *ad libitum*, but grain was limited to from 3 to 5 pounds daily, varying with the group concerned. All calves were fed similarly the first two weeks of age, being allowed to remain with the dams for approximately one day and fed whole milk until two weeks of age, at which time they were gradually changed to the experimental feed.

Group I was fed skimmilk at the rate of 12 to 16 pounds daily until six months of age. Group II was also fed skimmilk, but was limited to 12 pounds of skimmilk daily and milk feeding discontinued at four months of age. Groups III and IV were fed a solution of buttermilk curd ¹ at the rate of 12 to 16 pounds per calf daily. In Group IV the solution was discontinued when the calves were four months of age, whereas in Group III the buttermilk curd solution was fed throughout the six months' period. Group V was fed a solution of Hi-Lactic milk ² in a manner similar to that of Group III. Group VI was fed 12 pounds daily of a solution of powdered buttermilk, made up of 1.2 pounds of powder in 12 pounds of solution, until 35 days of age, when the solution was gradually replaced during one week's time by an equivalent amount of buttermilk powder, fed dry in the grain ration. Group VII was fed similarly excepting that 12 pounds of buttermilk solution contained 1.4 pounds of buttermilk powder. Group VIII was fed similarly to Group VII, except that powdered skimmilk was the experimental feed.

DISCUSSION OF RESULTS

The data have been summarized in two parts, one part dealing with water consumption of five groups of calves fed liquid milk and the other part with the water consumption of three groups of calves fed powdered milk as part of the grain ration after 35 days of age.

Average weekly water consumption per calf for the calves fed liquid milk is shown by weeks up to six months of age in table 1 and figure 1. Table 1 in addition to presenting total water subdivides water into free water, water in liquid milk, and water in hay and grain. Average pounds of dry matter consumed weekly per calf, water per pound of dry matter, average body weight, and water per 100 pounds of body weight are also presented in table 1. Since some of the calves did not receive milk to six months of age, the data presented as averages for the calves fed liquid milk represent 18 calves the first 16 weeks, 16 calves the seventeenth week, and 12 calves during the eighteenth to twenty-sixth weeks inclusive.

Water consumed in the form of hay and grain was of little importance. The maximum of seven pounds, or one pound daily, occurred in the twenty-sixth week. Milk was the primary source of water during the first few

¹ Buttermilk curd is similar in appearance to semisolid buttermilk, but contains more moisture. It is made by coagulating the buttermilk, placing coagulant in burlap sacks, and removing moisture by pressure.

² Hi-Lactic milk is a commercial concentrated sour skimmilk.

weeks of the calf's life, but represented a decreasing percentage of the total water intake as the calf became older because the amount of milk fed was purposely limited. When the calves were four weeks of age milk represented 99 per cent of the total water consumed; when 8 weeks, 81 per cent; 12 weeks, 61 per cent; 16 weeks, 47 per cent; 20 weeks, 41 per cent; and 26 weeks, 28 per cent. The quantity of water obtained from milk varied from 64 to 93 pounds, averaging 76 pounds per week, or 11 pounds daily per calf.

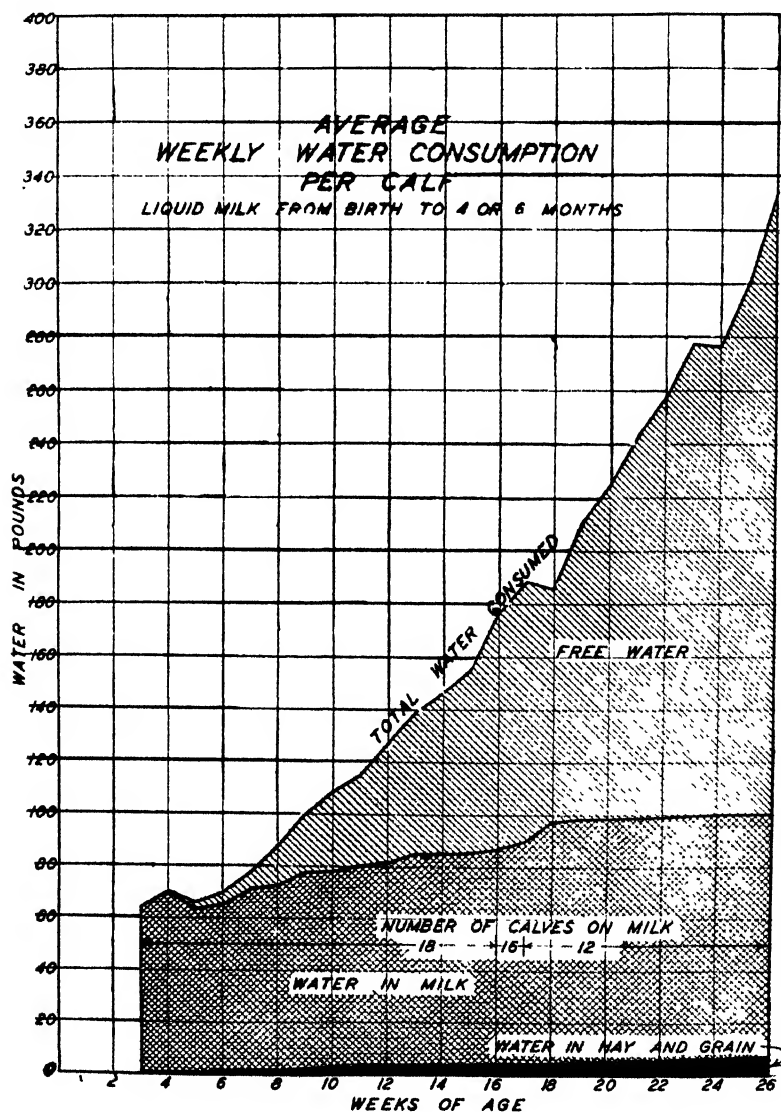


FIG. 1. AVERAGE WEEKLY WATER CONSUMPTION PER CALF FOR CALVES FED LIQUID MILK.

TABLE 1
Average weekly water consumption per calf when liquid milk was fed

AGE	FREE WATER	WATER IN MILK	WATER IN HAY AND GRAIN	TOTAL WATER	DRY MATTER	WATER PER POUND DRY MATTER	BODY WEIGHT	TOTAL WATER PER CWT. BODY WEIGHT
<i>weeks</i>	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>
3	0.1	63.9	0.2	64.2	10.0	6.4	106.8	60.1
4	0.5	69.2	0.5	70.2	11.7	6.0	121.3	57.9
5	1.7	64.0	0.7	66.4	12.3	5.4	129.2	51.4
6	3.8	65.2	1.1	70.1	18.0	3.9	135.7	51.7
7	7.0	71.2	1.5	79.7	23.1	3.5	147.4	54.1
8	15.3	71.2	1.8	88.3	25.9	3.4	157.7	56.0
9	22.4	75.7	2.3	100.4	30.9	3.2	168.0	59.8
10	30.2	75.8	2.6	108.6	34.5	3.1	181.1	60.0
11	34.4	77.4	3.0	114.6	36.6	3.1	192.2	59.7
12	46.2	78.2	3.2	127.6	39.1	3.3	204.7	62.3
13	54.4	81.6	3.3	139.3	40.6	3.4	217.9	63.9
14	61.5	81.6	3.5	146.6	43.1	3.4	229.8	63.8
15	70.4	81.5	3.9	155.8	48.5	3.2	242.4	64.3
16	90.4	82.6	4.0	177.0	49.6	3.6	256.3	69.0
17	98.7	85.8	4.3	188.8	51.3	3.6	277.9	67.9
18	88.1	190.3	4.4	185.9	194.7	3.6	288.3	64.5
19	113.1	217.1	4.9	211.4	222.0	3.7	303.5	69.7
20	126.7	247.1	5.1	225.2	252.2	3.9	317.5	70.9
21	146.0	233.8	5.3	243.8	239.1	4.0	337.0	72.3
22	157.5	250.9	5.9	256.8	256.8	4.0	351.1	73.1
23	177.5	270.6	6.4	277.3	277.0	3.9	369.6	75.0
24	176.6	284.4	6.5	276.5	290.9	4.0	387.5	71.4
25	200.9	297.8	6.7	301.0	304.5	4.1	407.0	74.0
26	233.6	257.0	7.0	334.0	264.0	4.1	423.1	78.9

The data represent averages for 18 calves that received liquid milk the first 16 weeks, 16 calves that received milk during the seventeenth week, and 12 calves that received milk from the eighteenth to twenty-sixth weeks inclusive.

*This column represents the two groups (6 calves) that received no milk after the seventeenth week.

Free water drunk weekly per calf averaged 0.5 of a pound at 4 weeks of age, 15.3 pounds at 8 weeks, 46.2 at 12 weeks, 90.4 at 16 weeks, 126.7 at 20 weeks, and 233.6 at 26 weeks.

Total water intake per calf weekly averaged 70.2 pounds at 4 weeks of age, 88.3 at 8 weeks, 127.6 at 12 weeks, 177.0 at 16 weeks, 225.2 at 20 weeks, and 334.0 at 26 weeks.

These results indicate that when liquid skim milk is fed free water is

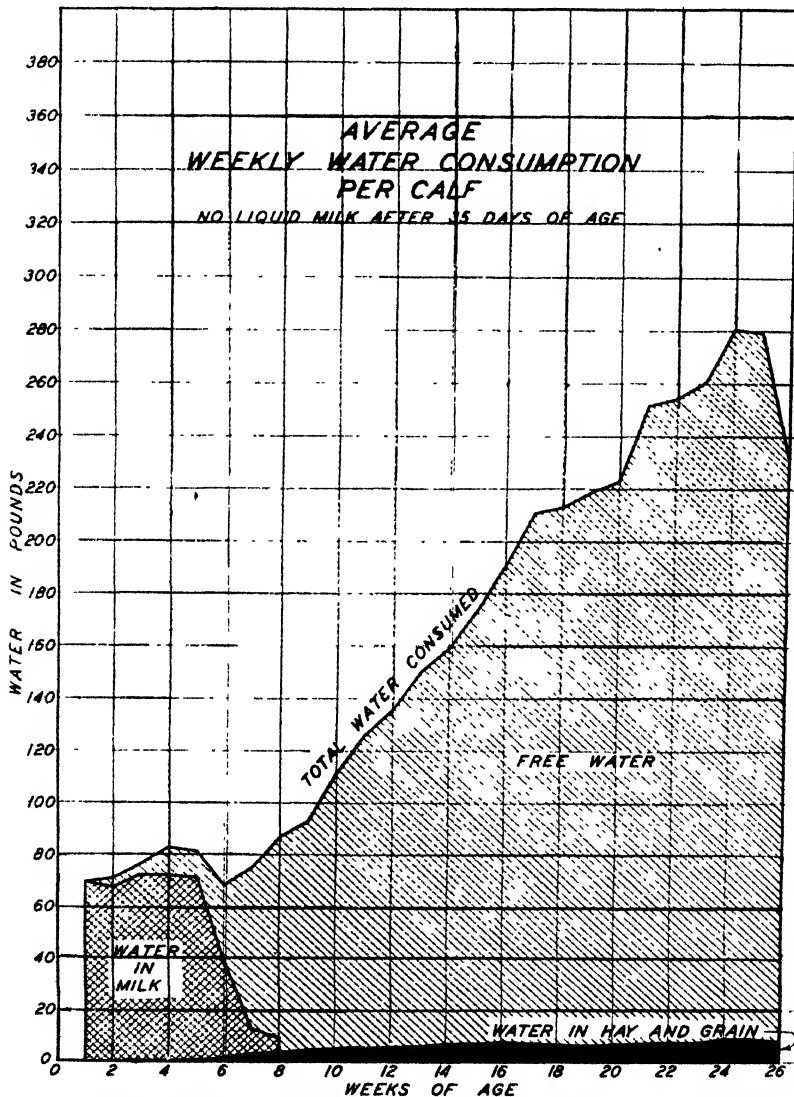


FIG. 2. AVERAGE WEEKLY WATER CONSUMPTION PER CALF FOR 12 CALVES WHEN NO LIQUID MILK WAS FED AFTER THE CALVES WERE 35 DAYS OLD. DRIED MILK WAS FED AS PART OF THE GRAIN RATION UNTIL THE CALVES WERE EITHER 4 OR 6 MONTHS OF AGE.

not important until the calf is at least 8 weeks of age since during the eighth week the calves drank an average of only 2.2 pounds of water daily. After the calves were two months old free water became increasingly more important as the calves became older, but previous to that age access to free water does not seem as essential as sometimes suggested. If the amount of milk fed daily were increased, the age when free water would be important would be extended. At what age the absence of free water would become a limiting factor cannot be determined from these experiments. The results are useful, however, in considering the normal total water requirements of calves at various ages by weeks up to six months when fed liquid milk.

Table 2 and figure 2 show the average weekly water consumption per calf for 12 calves fed milk powder as part of the grain ration, with no liquid milk after 35 days of age. In addition to total water table 2 presents water subdivided into free water, water in liquid milk, and water in hay and grain. Average pounds of dry matter consumed weekly per calf, water per pound of dry matter, average body weight, and water per 100 pounds of body weight are also presented in table 2.

TABLE 2
*Average Weekly Water Consumption Per Calf When Dried Milk
Was Fed in the Grain Ration
(Liquid milk was fed until the calves were 35 days of age)*

AGE	FREE WATER	WATER IN LIQUID MILK	WATER IN HAY AND GRAIN	TOTAL WATER	DRY MATTER	WATER PER POUND DRY MATTER	BODY WEIGHT	WATER PER CWT. BODY WEIGHT
<i>weeks</i>	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>
1		69.4		69.4	10.4	6.7	108.9	63.7
2	3.3	67.6	0.1	71.0	10.2	7.0	112.9	62.9
3	3.9	72.5	0.2	76.6	11.0	7.0	120.0	63.8
4	10.5	72.1	0.4	83.0	11.0	7.5	128.0	64.8
5	10.4	70.5	0.7	81.6	12.2	6.7	139.6	58.4
6	28.8	37.7	1.6	68.1	14.9	4.5	150.2	45.3
7	62.7	9.8	3.0	75.5	21.2	3.5	160.8	46.7
8	78.8	4.8	3.7	87.3	25.0	3.5	172.9	50.3
9	89.0		4.4	93.4	29.2	3.2	181.6	51.2
10	106.8		5.1	111.9	33.7	3.3	195.3	57.0
11	121.2		5.3	126.5	35.3	3.6	208.1	60.5
12	130.4		5.8	136.2	38.7	3.5	218.8	62.0
13	144.6		6.0	150.1	40.8	3.7	230.7	65.1
14	153.5		6.7	160.2	45.6	3.5	245.0	65.1
15	167.4		6.8	174.2	46.8	3.7	260.3	66.7
16	189.6		7.3	196.5	51.5	3.8	272.0	72.2
17	203.9		7.0	210.9	52.8	4.0	285.4	73.9
18	206.4		6.7	213.1	50.0	4.3	296.7	71.8
19	213.0		6.1	219.1	46.3	4.7	306.8	71.4
20	215.1		6.9	222.0	51.4	4.3	321.8	69.0
21	244.6		7.1	251.7	53.6	4.7	334.3	75.3
22	246.5		7.8	254.3	59.5	4.3	344.6	73.8
23	252.9		7.8	260.7	59.8	4.4	359.4	72.5
24	271.7		8.7	280.4	66.5	4.2	373.7	75.0
25	270.4		8.7	279.1	67.1	4.2	388.2	71.9
26	223.9		8.0	231.9	62.2	3.7	399.7	58.0

The data represent averages for 12 calves.

Little free water was consumed while liquid milk was fed, as might be expected from figure 1, since these calves were taken off liquid milk before free water consumption became important. Change from liquid milk to the dry grain mixture caused a temporary reduction in total water consumption, due to the fact that the calves did not adjust themselves to the change immediately. After these calves had been on the dry grain mixture for two weeks their water consumption equalled that of the liquid milk group and continued to increase as the calves grew older. Except for the adjustment period, total water intake in these groups was approximately the same as for the groups fed liquid skim milk. Enough additional free water was consumed to compensate for the water normally consumed in liquid milk.

Further evidence that free water consumption depends on the character of the ration is found in table 1 for the two groups of calves which received no milk after the seventeenth week. Enough more free water was drunk to make the total water intake about the same as for those calves receiving milk up to six months of age.

These facts indicate that the total water requirements of calves at the various ages are rather definite, regardless of the form in which the water is consumed.

These results are in accord with the report of Atkeson and Warren (1) on the total water consumption of dairy cows. They found that total water intake was rather constant, other conditions being controlled, regardless of whether or not succulent feed was fed, the adjustment being made by the cows through more or less free water drunk.

Figure 3 shows the relationships of total water intake, body weight, and dry matter consumed for the calves receiving liquid milk (table 1). Considering the fact that the season of year when the calves were born resulted in an increase in temperature as the calves became older, the relationship between total water intake and body weight and dry matter consumed is quite marked. During the first 10 weeks when the calves' diet was principally milk, the relationship between dry matter and water was not so close. The relationship between dry matter consumed and body weight was quite constant from the tenth to the twenty-sixth week. This is of particular interest since hay was fed at will and although an upper limit was established for grain the amount fed varied with the condition of the individual calves. Quite similar results were obtained with the calves fed no liquid milk after 35 days of age (table 2). Whether total water intake depends on body weight or on dry matter consumed or both cannot be determined from these results because of the constant relationship between body weight and dry matter consumed. Recent work by Atkeson and Warren (1) indicates that with non-lactating dairy cows dry matter consumption seems to govern total water intake.

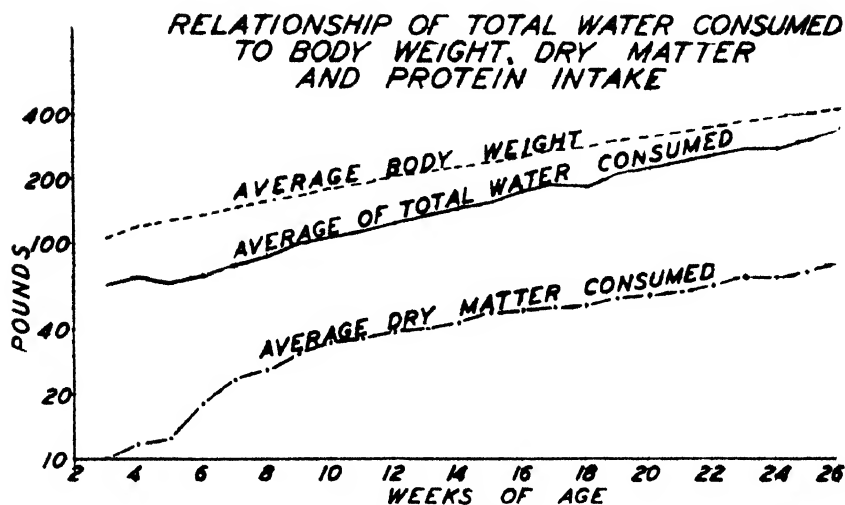


FIG. 3. RELATIONSHIP OF AVERAGE TOTAL WATER CONSUMED TO AVERAGE BODY WEIGHT AND TO AVERAGE DRY MATTER CONSUMED FOR THE CALVES FED LIQUID MILK UP TO 4 OR 6 MONTHS. DATA FROM TABLES 1 AND 2 ARE CHARTED SEMI-LOGARITHMICALLY TO FACILITATE COMPARISONS.

CONCLUSIONS

The total water requirement of dairy calves seems to be rather definite at various ages up to six months. Free water does not seem important for calves receiving liquid milk until they are at least eight weeks of age, but becomes increasingly more important as the calves become older. When liquid milk is removed from the diet, calves tend to drink enough more free water to compensate for the water in the milk, thereby keeping the total water intake about the same as for calves fed liquid milk. The relationship between total water intake, body weight, and dry matter consumption was quite constant, especially after the tenth week.

REFERENCES

- (1) ATKESON, F. W., AND WARREN, T. R. The influence of type of ration and plane of production on water consumption of dairy cows. *JOUR. DAIRY SCI.* 17: 265-277. 1934.
- (2) BOWLING, G. A., AND ACKERMAN, R. A. Water vs. no water in the feeding of dairy calves when on skim milk. Abstracts of papers presented at the twenty-eighth annual meeting of the American Dairy Science Assoc., p. 72. 1933.
- (3) FULLER, J. M. Some physical and physiological activities of dairy cows. *New Hampshire Agr. Expt. Sta. Tech. Bul.* 35, pp. 9-10. 1928.
- (4) HENRY, W. A., AND MORRISON, F. B. Feeds and feeding, 18th edition, p. 428. The Henry-Morrison Publishing Co., Madison, Wisconsin. 1923.
- (5) McCANDLISH, ANDREW C. The feeding of dairy cattle, p. 224. John Wiley and Sons, New York. 1922.
- (6) RITZMAN, E. G., AND BENEDICT, F. H. The effect of varying feed levels on the physiological economy of steers. *New Hampshire Agr. Expt. Sta. Tech. Bul.* 26, pp. 20-21. 1924.
- (7) WALL, F. W. Productive feeding of farm animals, p. 222. J. B. Lippincott Co., Philadelphia, Pa. 1915.

INFLUENCE OF MASTITIS ON THE CURD TENSION OF MILK*

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Considerable interest has developed in soft curd milk since Hill (5) developed a test for quantitatively measuring the difference in curd character of milk from individual cows and indicated the value of soft curd milk for infant feeding. Hill (7) calls any milk with a curd tension of less than 30 grams "soft curd milk" and all above 30 grams "hard curd milk." This standard has been adopted by various health departments which are making soft curd determinations.

Hill (6, 7, 8) and Espe and Dye (4) have found that soft curd milk is more easily digested than hard curd milk and that cow's milk with the soft curd character is an excellent infant food. Weisberg, Johnson, and McCollum (10) report that the concentration of casein is the major factor in determining the curd character of cow's milk and that soft curd milk contains less calcium and phosphorus than hard curd milk, but the ratio of calcium to phosphorus is substantially the same regardless of the curd character.

Allemann and Schmid (1), Buckley (2), and Hill (6, 7, 8) have established that curd tension varies with individual cows, stage of lactation period, and breeds.

Although both heat and homogenization, according to Hill (8), reduce the curd tension of milk, the prevailing method of producing soft curd milk is to select milk from cows naturally producing it. Observation in the University of Idaho dairy herd and in commercial dairy herds indicated some relationship between mastitis and the production of soft curd milk.

Mastitis is one of the most prevalent diseases of dairy cattle. Most of the authorities on mastitis are agreed that a large majority of the cases are caused by a streptococcic infection. In some instances the organism causing mastitis, if of human origin, has been responsible for epidemics of septic sore throat. If there is any relationship between mastitis and the production of soft curd milk by individual cows, it is obvious that greater precautions must be taken in the production of this type of milk.

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PROCEDURE

Milk used in this investigation came from individual cows of the University of Idaho dairy herd, composed of Holsteins and Jerseys. This herd is under constant veterinary supervision and is federally accredited for tuberculosis and state accredited for Bang's abortion disease.

Part I

A composite sample was taken from each cow's milk, after discarding the fore milk, and the following determinations made:

1. Bacterial count by plate method according to "Standard Methods of Milk Analysis."
2. Bacterial count by plate method, using nutrient agar to which had been added one per cent of dextrose and five per cent of defibrinated horse blood.
3. Leucocyte count, using the Prescott and Breed technic for direct count.
4. Curd tension, using the procedure and equipment recommended by Hill (7).
5. Streptococci or staphylococci grouping.

Part II

Samples of milk were taken from each individual quarter of each of the 12 cows used. A special milk pail with four separate compartments was used. Milk from each quarter was weighed and sampled. After sampling, the milk from each quarter was thoroughly mixed with the milk from the other three quarters and a composite sample taken as a check against the individual quarters. The same tests were made as in Part I, and, in addition, the black cloth test for detecting the presence of white flakes in the milk.

A physical examination of the udders of all cows was made the same day samples were taken to determine physical symptoms indicative of mastitis.

All cases of mastitis presented in this paper were chronic in nature.

PRESENTATION OF DATA

Part I

Table 1 presents the results on 46 composite samples of milk from 26 individual Holsteins and 20 individual Jerseys. Results show that with the occurrence of mastitis there was an increase in bacterial counts on both plain and blood agar, an increase in leucocyte counts, and a decrease in curd tension.

TABLE 1

*Influence of mastitis on bacterial count, leucocyte count, and curd tension of milk
(Composite Samples)*

NO. OF COW	BACTERIA PER CC.		LEUCOCYTES PER CC.	CURD TENSION
	Plain agar	Blood agar		
Normal Cows				
64	100	4,200	93,000	53.0
74	700	9,200	93,000	26.0
2X	400	1,000	70,000	47.5
4X	300	200	23,000	26.0
5X	400	400	46,000	33.5
8X	300	200	46,000	37.5
9X	500	400	46,000	52.5
19X	100	100	23,000	37.5
22X	500	100	46,000	40.0
23X	700	400	46,000	30.0
25X	50	300	93,000	50.0
26X	200	400	23,000	28.5
27X	1,000	800	11,500	23.5
28X	50	100	11,500	30.0
155	3,200	2,000	23,000	94.0
156	300	100	46,000	50.0
157	1,600	3,200	132,000	62.0
160	800	300	23,000	70.0
165	50	50	46,000	60.0
168	300	400	46,000	52.5
169	50	100	23,000	82.0
170	200	100	23,000	84.5
172	1,400	1,800	46,000	74.0
173	200	0	132,000	80.0
175	500	50	23,000	70.5
176	100	100	46,000	72.0
178	800	100	23,000	55.0
181	2,700	2,100	11,500	42.5
198	0	100	11,500	55.0
Streptococcie Infection				
55	300	500	300,000	28.0
65	7,000	6,200	8,800,000	24.0
67	650	2,000	308,000	32.5
83	10,000	13,000	2,040,000	15.0
78	100	225,000	2,420,000	14.0
98	100	42,000	934,000	23.0
18X	8,000	10,600	880,000	20.0
24X	75,000	240,000	140,000	19.0
52	8,400	24,000	1,868,000	10.0
151	10,200	76,000	8,000,000	62.0
164	600	500	640,000	40.0
167	200	4,200	1,760,000	23.0
Staphylococcie Infection				
70	1,800	5,400	46,000	36.0
80	4,200	300	280,000	67.5
13X	3,000	15,000	858,000	37.5
150	1,200	8,000	2,040,000	37.5
159	1,500	1,750	640,000	87.0

Note: Cows with numbers below 100 are Holsteins and cows with numbers above 100 are Jerseys.

Of the 15 cows that produced soft curd milk, according to Hill's classification, 9, or 60 per cent, had chronic mastitis, all caused by a streptococcic infection. Each of these 9 cows produced milk having a leucocyte count in excess of 100,000, which agrees with Cherrington *et al.* (3), and in 8 of 9 instances, particularly on blood agar, a relatively high bacterial count.

Seventeen of the 46 cows had chronic mastitis. Twelve of the 17 cases of mastitis were caused by streptococcic infection and 5 by staphylococcic infection. The curd tension of the milk from cows with streptococcic infection ranged from 10 to 62 grams, with an average of 26.0 grams. Nine of these 12 cows produced milk with a curd tension below 30 grams. The curd tensions of milk from cows with staphylococcic infection were all above 30 grams and ranged from 36 to 87 grams, with an average of 53.0 grams. This would indicate that mastitis caused by a streptococcic infection lowers the curd tension more than mastitis caused by a staphylococcic infection.

Part II

When the relationship between mastitis and curd tension became apparent, it was thought advisable to continue the work under more carefully controlled conditions. All the milk from individual quarters was used and composite samples were not taken except as checks. Two preliminary trials were run, followed by a third trial in which detailed information was secured.

Table 2 presents the data of the third trial on individual quarters of 12 cows, 6 Holsteins and 6 Jerseys. Two cows of each breed were normal, healthy cows; and four of each breed had chronic mastitis in one or more quarters.

In every instance milk from quarters having mastitis showed a higher leucocyte count than milk from normal quarters and in 7 out of 9 instances a lower curd tension. Every quarter having a streptococcic infection gave milk with a curd tension of 30 grams or below, while quarters having a staphylococcic infection gave milk with a high curd tension.

The curd tension of milk from individual quarters of normal cows was comparatively uniform. However, when one or more quarters of cows having mastitis had a streptococcic infection, the curd tension of these quarters was lower than that for normal quarters.

The black cloth test was very effective in determining mastitis caused by a streptococcic infection. Streptococcic infection usually caused flaky milk, whereas staphylococcic infection did not.

Data in table 2 do not seem to show any correlation between the development of fibrous tissue in a quarter and low curd tension of milk from that quarter. Five quarters showed a slight fibrous development, only one of which showed lower curd tension than the average from the cows involved. Six quarters showed moderate to extensive fibrous tissue development, three

TABLE 2
Influence of mastitis on bacterial count, leucocyte count, and curd tension of milk
(Individual Quarters)

NO. OF COW	QUARTER	BACTERIA PER CC.		LEUCOCYTES PER CC.	BLACK CLOTH TEST	TYPE OF INFECTION	VETERINARY EXAMINATION OF UDDER	CURD TENSION
		Plain agar	Blood agar					
8X	R.F.	9,000	7,800	23,000	-	Normal Cows	Very meaty	26.0
	R.R.	100	500	23,000	-		Very meaty	26.0
	L.R.	600	300	23,000	-		Very meaty	26.0
	L.F.	400	1,100	46,000	-		Meaty	22.0
Composite	R.F.	900	500	46,000	-		Meaty, extra heavy, sl. fibrosa	22.0
	R.R.	2,200	900	23,000	-		Meaty	33.0
	L.R.	800	2,600	23,000	-		Meaty	32.0
	L.F.	300	600	46,000	-		Meaty	30.0
Composite	R.F.	900	1,300	23,000	-		Meaty	32.0
	R.R.	3,500	2,600	46,000	-		Normal	33.0
	L.R.	7,600	2,400	23,000	-		Normal	60.0
	L.F.	17,600	10,300	23,000	-		Left side light	61.0
165	R.F.	7,300	4,800	23,000	-		Left side light	70.0
	R.R.	8,000	4,300	46,000	-		Normal	63.5
	L.R.	1,600	800	23,000	-		Normal	111.0
	L.F.	3,300	1,800	23,000	-		Normal	122.5
Composite	R.F.	4,000	1,700	23,000	-		Normal	93.5
	R.R.	4,800	1,900	23,000	-		Normal	98.5
	L.R.	3,600	1,600	23,000	-		Normal	95.0
	L.F.				-			
55	R.F.	5,200	5,600	960,000	+	Streptococci Infection	Udder very meaty, Fibrosa 1	19.0
	R.R.	5,000	9,800	184,000	+		Udder very meaty	23.0
	L.R.	9,600	10,300	92,000	-		Udder very meaty, Fibrosa 1	28.5
	L.F.	11,400	13,200	46,000	-		Udder very meaty	26.0
Composite	R.F.	11,600	13,200	460,000	+		Meaty, spider	23.5
	R.R.	1,400	16,200	230,000	-		Meaty, spider	27.0
	L.R.	2,400	23,000	460,000	+		Meaty, spider	25.0
	L.F.	300,000	275,000	322,000	+		Meaty, spider	25.0
24X	R.F.	7,200	10,000	138,000	-		Meaty, spider	24.0
	R.R.	36,000	43,000	322,000	+		Meaty, spider	24.0
	L.R.				-			
	L.F.				+			
Composite	R.F.				+			
	R.R.				+			
	L.R.				-			
	L.F.				+			

TABLE 2—(Continued)

NO. OF COW	QUARTER	BACTERIA PER CC.		LEUCOCYTES PER CC.	BLACK CLOTH TEST	TYPE OF INFECTION	VETERINARY EXAMINATION OF UDDER	CURD TENSION
		Plain agar	Blood agar					
67	R.F.	10,000	16,000	312,000	+	Streptococcic	Meaty	30.0
	R.R.	10,400	12,400	276,000	-	Streptococcic	Meaty	35.0
	L.R.	21,000	20,000	920,000	-	Streptococcic	Meaty	20.0
	L.F.	8,000	10,200	276,000	-	Streptococcic	Meaty	30.0
Composite		14,400	17,300	322,000	+	Streptococcic		30.0
167	R.F.	100	1,000	1,186,000	-	Streptococcic	Udder meaty	28.0
	R.R.	100	200	467,000	-	Streptococcic	Udder meaty	54.0
	L.R.	100	100	92,000	-	Streptococcic	Udder meaty	56.0
	L.F.	0	100	186,000	-	Streptococcic	Udder meaty	46.0
Composite		200	300	794,000	-	Streptococcic		49.0
151	R.F.	1,000	3,400	966,000	-	Streptococcic	Fibrosa 1	75.0
	R.R.	8,000	9,700	1,610,000	-	Streptococcic	Fibrosa 2	55.0
	L.R.	18,200	33,000	6,900,000	+	Streptococcic	Fibrosa 3	25.0
	L.F.	3,200	4,000	96,000	-	Streptococcic	Fibrosa 3	67.5
Composite		3,600	3,900	736,000	+	Streptococcic		67.5
70	R.F.	800	2,100	276,000	-	Staphylococcic Infection	Normal	47.5
	R.R.	6,200	9,000	23,000	-	Staphylococcic	Normal	50.0
	L.R.	1,900	1,000	23,000	-	Staphylococcic	Slight fibrosa, light quarter	47.5
	L.F.	1,300	1,000	46,000	-	Staphylococcic	Light quarter	40.0
Composite		2,300	3,100	92,000	-	Staphylococcic		47.5
150	R.F.	8,000	9,200	46,000	-	Staphylococcic	Shrunk	61.0
	R.R.	110,000	100,000	46,000	-	Staphylococcic	Normal	72.5
	L.R.	5,200	6,000	3,036,000	-	Staphylococcic	Fibrosa 3	47.0
	L.F.	12,000	14,000	92,000	-	Staphylococcic	Fibrosa 1	74.0
Composite		16,000	19,500	276,000	-	Staphylococcic		74.0
159	R.F.	0	200	327,000	-	Staphylococcic	Normal	111.0
	R.R.	100	400	187,000	-	Staphylococcic	Fibrosa 2	110.0
	L.R.	1,400	1,700	233,000	-	Staphylococcic	Fibrosa 3	98.0
	L.F.	400	600	140,000	-	Staphylococcic	Normal	95.0
Composite		600	700	280,000	-	Staphylococcic		106.0

of which showed a curd tension lower than the composite sample from the cow involved. Meaty or coarse udders showed no more tendency to low curd tension milk than fine-textured udders.

DISCUSSION OF RESULTS

Soft curd milk is, in general, produced only by the more modern and better equipped dairies. These dairies have constant and competent veterinary inspection and supervision. The practice is to immediately remove from the herd any cow contracting mastitis. Under these conditions there is little likelihood of any soft curd milk being produced by cows having mastitis. However, due to competition some dairies not so efficiently managed and without regular veterinary service may attempt to produce soft curd milk. Without regular veterinary service the production of soft curd milk holds much danger since it is frequently difficult to determine the presence of mastitis. Data presented in this paper indicate that if mastitis is caused by a streptococcic infection the milk will invariably have a lower curd tension than milk from either normal quarters or normal cows. The streptococcic infection usually lowers the curd tension sufficiently for the milk to be called "soft curd milk." However, if the mastitis is caused by a staphylococcic infection there does not seem to be any appreciable effect on the curd tension. In this work the black cloth test was very successful in indicating mastitis when caused by a streptococcic infection but not when caused by a staphylococcic infection.

Udall and Johnson (9) have stated that a physical examination of the udder for excessive fibrous tissue immediately following milking is a reasonably satisfactory method of detecting mastitis. Since it appears that mastitis usually lowers the curd tension of milk, there might logically be expected some relationship between the development of fibrous tissue in a quarter and low curd tension of the milk from that quarter. Data presented in table 2 do not substantiate that hypothesis.

It might appear that undue emphasis has been placed on the possibility of soft curd milk's being caused by mastitis. It is true that in certified dairies this possibility is remote, but in most herds mastitis occurs frequently. It is apparent from the data presented that chronic mastitis caused by a streptococcic infection might cause milk from individual cows to be of a soft curd character. In fact, the possibility is so great that it is recommended that no cow be used for the production of soft curd milk unless it is absolutely certain that she is at all times free from mastitis as determined by both a physical and bacteriological examination.

SUMMARY

1. Mastitis caused by a streptococcic infection invariably lowered the curd tension of milk.

2. Mastitis caused by a staphylococcic infection apparently had no appreciable influence on the curd tension of milk.

3. The black cloth test successfully detected mastitis caused by a streptococcic infection, but not that caused by a staphylococcic infection.

4. There was no correlation between the development of fibrous tissue in the udder and the curd tension of the milk in the cases studied.

Since this paper was accepted for publication a similar paper entitled "Soft Curd Milk and Mastitis," by R. C. Welch and F. J. Doan has been published in the *Milk Plant Monthly*, vol. 22, No. 11, p. 30-36, Nov. 1933. The latter was contributed from the Pennsylvania Agricultural Experiment Station as Technical Paper No. 608, approved for publication Oct. 7, 1933.

REFERENCES

- (1) ALLEMAN, O., and SCHMID, H. Concerning the solidity of milk coagulones produced by rennet. *Landwirthsches Jahrbuch der Schweiz* 30: 355-381. 1916.
- (2) BUCKLEY, S. S. The physical character of the curd of milk from different breeds; curd as an index of the food value of milk; studies of the proteid contents of milk. Bulletin 184, Maryland Agr. Expt. Sta. 1914.
- (3) CHERRINGTON, V. A., HANSEN, H. C., and HALVERSEN, W. V. The leucocyte content of milk as correlated with bacterial count and hydrogen ion concentration for the detection of mastitis. *JOUR. DAIRY SCI.* 16: 59-67. 1933.
- (4) ESPE, D. L., and DYE, J. A. Effect of curd tension in the digestibility of milk. *Amer. Jour. Dis. Child.* 43: 26. 1932.
- (5) HILL, R. L. The test for determining the character of the curd from cow's milk and its application to the study of curd variance as an index to the food value of milk for infants. *JOUR. DAIRY SCI.* 6: 509-526. 1923.
- (6) HILL, R. L. The physical curd character of milk and its relationship to the digestibility and food value for infants. Bulletin 207, Utah Agr. Expt. Sta. 1928.
- (7) HILL, R. L. Soft-curd milk. Bulletin 227, Utah Agr. Expt. Sta. 1931.
- (8) HILL, R. L. A decade and a half of soft-curd milk studies. Circular 101, Utah Agr. Expt. Sta. 1933.
- (9) UDALL, D. H., and JOHNSON, S. D. The diagnosis and control of mastitis. *Cornell Veterinarian* 21: 190-206. 1931.
- (10) WEISBERG, S. M., JOHNSON, A. H., and MCCOLLUM, E. V. Laboratory studies on the chemistry of soft curd milk. *JOUR. DAIRY SCI.* 16: 225-247. 1933.

American Dairy Science Association Announcements

WESTERN DIVISION

The proceedings of the meeting of the Western Division of the American Dairy Science Association have been mimeographed in full, including all papers presented. The meeting was held in the Multnomah Hotel in Portland, Oregon, on October 22, with G. H. Wilster presiding.

In the manufacturing section papers were presented on raw versus pasteurized milk for brick cheese by A. J. Morris, on chlorine sterilization by C. S. Mudge, on mastitis and curd tension by H. C. Hansen, on butter quality by J. A. Nelson, on vitamin D milk by C. L. Roadhouse, and on dairy farm milk coolers by Hans Hoffman. In the production section papers were presented on artificially dried pasture herbage by J. C. Knott and R. E. Hodgson, on grazing habits of dairy cows by R. E. Hodgson, on some unsolved feeding problems by I. R. Jones, on the need for simplification of testing dairy cows for production by E. V. Ellington, on the extension program by D. L. Fourn, and on new ideas for dairy cattle shows by H. M. King.

The Western Division sponsors a students' judging contest at the Pacific International Livestock Exposition. First place in judging all dairy products was won by Utah, second place by Washington, and third place by Montana. In judging all breeds of dairy cattle first place was won by Washington, second by Idaho, and third by Oregon.

METHODS OF ANALYZING DAIRY PRODUCTS

The methods of testing dairy products which were recently published in the Journal may still be secured from R. S. Breed, Geneva, N. Y., at 50 cents per copy.

STANDARD METHODS OF MILK ANALYSIS

The American Public Health Association announces the sixth edition of Standard Methods of Milk Analysis. Many important additions and changes occur.

In view of the extensive alterations and additions, it is urged that the Fifth Edition published in 1927 be discarded. The new edition may be purchased from the American Public Health Association, 450 Seventh Avenue, New York, N. Y., for \$1.00.

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LABORATORY METHODS FOR THE DETECTION OF MILK FROM COWS INFECTED WITH MASTITIS

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The common occurrence of udder infections in dairy cows has motivated investigators to develop reliable laboratory methods for detecting milk which has been produced wholly or in part by animals suffering from such an infection.* It is not the purpose of this paper to discuss the public health aspects of consuming milk from diseased udders, but merely to present data and discussion which may assist in determining the reliability of available methods for detecting milk produced by cows suffering from this condition. The data presented in this paper were secured from a large herd under efficient management. The cases of mastitis reported are usually of the sub-clinical or chronic type which are so prevalent and may exist for years without the dairyman's knowledge. However, occasional cases of acute mastitis occurred and these were also included.

HISTORICAL

The prevalence of chronic streptococcic mastitis in cows can well be judged by the report of Rosell (24), who has found that it affects from 20 to 50 per cent of high milk producers in different countries, and causes a greater loss to the milk industry than any other cattle disease. He found that chronic streptococcic mastitis actually constitutes at least 98 per cent of all cases in high-producing cows.

Methods employed for the detection of milk from infected udders have generally been developed on the basis of (1) direct microscopic examination of milk to determine the presence of abnormal cellular constituents—streptococci, leucocytes, fixed tissue cells, etc.; (2) cultural tests to reveal the numbers and kinds of bacteria present; and (3) chemical tests which would reveal the presence of abnormal constituents in the milk. It is not the intention of this paper to present a complete bibliography on the various phases of this subject, but merely to cite a few pertinent references which will indicate some of the previous results and methods of attack.

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The direct microscopic bacterial examination of milk for detecting udder infection is necessarily limited to freshly drawn samples from individual cows. In cases where the infection is not extremely severe it would probably be necessary to use samples from the individual quarters. Breed and Brew (5) recommended the microscopic examination of milk as a means of detecting high counts due to udders infected with streptococci.

The occurrence of an abnormal number of leucocytes and fixed tissue cells in milk has also been considered as an indication of infection. Opinion differs on this subject; for example, Breed (3, 4) reported obtaining apparently normal milk strippings from a single teat which contained 54,000,000 leucocytes per cubic centimeter; no streptococci or other undesirable bacteria could be found in this milk. In contrast to these findings Cherrington *et al.* (7), using the Prescott and Breed (23) method, concluded that "Milk from normal udders usually contains less than 50,000 leucocytes per cubic centimeter, whereas milk from infected udders almost invariably contains more than 100,000 leucocytes per cubic centimeter."

More recently Hucker *et al.* (12) studied the incidence of sub-clinical mastitis in dairy districts as well as methods of detecting this condition in 221 cows condemned by the tuberculin test. In their excellent report they conclude that "More than 3,000,000 cells per cubic centimeter almost invariably indicate a present or past infection with streptococci." In a more recent article Hucker (13) says "Ninety per cent of the normal quarters, *i.e.*, quarters free from scar tissue, showed less than 60,000 cells per cubic centimeter, while a great majority, *viz.*, 87 per cent, never gave a higher count than 30,000 cells per cubic centimeter."

Cultural tests generally have been used to determine the sanitary quality of market milk samples. Samples of milk used to determine the presence of udder infections should be taken aseptically from individual cows and cultured immediately. Hastings and Hoffman (9) studied two cows which produced milk containing large numbers of bacteria and leucocytes. They believed the animals had suffered from mastitis at some previous time and had become chronic carriers of bacteria. This probably explains the origin of the so-called "high count" cows. Cherrington *et al.* (6) present data showing that "bacterial counts pertaining to healthy cows show that 86 per cent of the counts on plain agar and 78 per cent on blood agar were less than 1,000 per cubic centimeter, whereas 69 per cent of the samples from infected udders contained more than 2,000 bacteria per cubic centimeter on either plain or blood agar." They conclude that "Bacterial counts on blood agar were more reliable for detecting udder infections than those on plain agar because plain agar often failed to produce distinct colonies from the organisms which were responsible for the infection."

Hucker *et al.* (12) used not only the cell count as mentioned above but also "number of colonies appearing on veal infusion agar with and with-

out blood, number of colonies appearing on Burri slants, a direct microscopic examination, microscopic examination subsequent to incubation over night at 37° C., the amount of chlorides, lactose and catalase present and the reaction (brom-thymol-blue)."

Various modifications of the fermentation and curd tests have been used on market milk but they apparently never have been studied with the view of adapting them to the detection of udder infections. Orla-Jensen (22) suggests the rennet test to determine if milk has been drawn from diseased udders. More recent work by Hill (11) on the curd characteristic of milk shows that extreme variation exists between the curd characteristics of milk from individual cows within a given herd.

Chemical tests which would reveal the presence of substances present in milk from infected udders yet lacking in normal milk have been sought extensively. These tests generally have failed because of the complex composition of normal milk. Rosell (24) reports that the most important chemical changes in milk as the result of infection are (a) an increased pH, (b) an increase in catalase due to an increase in leucocytes, (c) increase in chlorine, (d) increase in heat coagulable albumen, and (e) decrease in potential acidity, lactose, casein, etc. He says "their determination is just as important as a bacteriological examination."

Baker and Van Slyke (2), Baker and Breed (1), Tweed (25), Hucker *et al.* (12), and Udall and Johnson (26) found a close relationship between decreasing hydrogen-ion concentration of the fresh milk and an abnormal number of leucocytes. They attribute the decreasing hydrogen-ion concentration to the entrance of the alkaline substances of the blood into the milk. The amount of chlorides in milk has been considered significant by Hammer & Bailey (8), Hayden (10) and Hucker (12). Hucker (14) summarizes: "Under general conditions it is assumed that all milk showing more than 0.14 per cent chlorides has been derived from infected udders." Hayden (10) developed several rapid tests for detecting milk which contained more than 0.14 per cent chlorine.

Enzymes also have been studied—oxidases and reductases and the very common catalase. The common occurrence of oxidases in normal milk precludes its application. However, the action of reductase has been employed in the well-known methylene blue test. This method has been used extensively for the grading of market milk, but its value for detecting milk from infected udders never has been adequately determined. Catalase, which occurs in abundance in blood and the tissues of the body, is present only to a slight degree in normal milk. Since infections liberate constituents of the blood into the milk in such quantities that the hydrogen-ion concentration is appreciably altered, it should only be expected that such milk serum mixtures would have an abnormal catalase content. Orla-Jensen (22) states, "Milk drawn from cows with diseased udders or from cows

which are approaching the end of the lactation period or colostrum, will liberate large amounts of oxygen from hydrogen peroxide." He employed a catalase test which he interpreted as a catalase number. He said fresh milk from healthy cows would not yield more than 2.5 cubic centimeters of oxygen from a mixture of 15 cubic centimeters of milk and sufficient hydrogen peroxide (1 to 3 per cent) to fill a 20 cc. test-tube. The number of cubic centimeters of gas produced at 20 degrees to 25 degrees Centigrade in six hours was taken as the catalase number. Hucker (12) used the catalase test and after stating that the test was very sensitive, concluded "that it is neither more delicate nor more accurate than other tests which are more easily made." Extensive studies on the catalase test have been made by Morgulus, Beber, and Rabkin (18, 19, 20), who point out the lack of knowledge of the theory of catalase activity.

A simple, quick, qualitative or quantitative colorimetric method for detecting pus in milk would be of great service for routine examination. Clinical methods have been developed for the detection of pus in urine, etc. These tests are usually based on the microscopic demonstration of large numbers of leucocytes. However, in cases where it is impossible to make a microscopic examination, chemical tests have been applied. Vitali's test and Donne's test have been used extensively for the detection of pus in urine. It would appear that the successful application of these tests to the detection of pus in urine would justify efforts to apply these methods to the detection of pus in milk. Their application to milk, however, is rendered difficult because of its complex composition. Kastle (15), in reviewing the chemical tests for blood, cites references which indicate that it is the iron of the hemoglobin and its iron-containing derivatives that are responsible for the guaiacum (Vitali's) reaction. He lists pus as a substance that does not react with guaiacum or other chromogenic substances employed in hematologic investigations, either alone or with hydrogen peroxide. Other chromogenic substances listed by Kastle that have been employed for this test are guaiacomic acid, aloin, benzidin, the leuco-base of malachite green, and phenolphthalein.

The constituents of pus serum may serve also as an index of pyogenic infections. These might include degradation products of bacteria, fixed tissue cells, leucocytes, or serum. Mathews (17) says, "There are in nearly all cells, and in possibly all cells, autolytic enzymes (endocellular enzymes) or nucleases, which decompose nucleic acids into its various constituents." Wells (29) describes the autolytic action taking place in necrosis, decomposing nucleoproteins of the nuclei and liberating nucleic acids. Later the nucleic acids are further decomposed into purine bases. Von Fellenberg (27) in reporting on the determination of purine bases in foods showed that animal products such as fish, poultry, beef, etc., as well as organs and glands were richest, while blood was poorer and milk and

practically free. Mandel and Levine (16) show that glucothionic acid derived from leucocytes is also present in pus. Wells (28) states that pus also contains other constituents of the leucocytes, particularly lecithin, cholesterol, fats (and soaps), cerebrin, "jecorin," and glycogen, and also the usual components of the blood serum as well as some small quantities of pigment derived from decomposed red corpuscles. The authors have found no references where tests based on these principles have been employed for the detection of pus in milk. Experiments are now under way with the hope of applying these principles.

PURPOSE OF THIS INVESTIGATION

The purpose of this investigation is to determine the merits of available methods for the detection of milk from infected udders. This study was undertaken with the hope of finding methods which would have sufficient merit to justify their adoption by dairy inspectors and laboratory technicians for routine testing of milk. Emphasis has been placed on the chemical composition of milk because of the desirability of having a quick chemical method that may be used by dairymen and inspectors with limited laboratory facilities. The methods should have sufficient reliability to detect the less severe cases of udder infection.

The cows used in this experiment belong to the dairy herd of the University of Idaho. They are free from tuberculosis and Bang's abortion disease as attested by repeated tests.

METHODS

Only middle milk was used in the following tests because of the wide variations in the numbers of bacteria generally reported in fore milk and strippings.

The numbers of leucocytes in milk were determined by the direct count method developed by Prescott and Breed (23). This method has been emphasized in a previous publication (7).

Enzymatic products were studied by making such tests as would demonstrate the presence of catalase and the oxidizing enzymes. The catalase test was run after the method described by Orla-Jensen (22). The guaiac test and the benzidin test for oxidizing enzymes were made according to standard clinical methods. The phenolphthalein test, which is also an oxidase test, was made according to the method of Kastle (15).

The presence of serum was demonstrated by determining the hydrogen-ion concentration of the milk with the quinhydrone electrode and the Brom cresol purple method of Baker and Van Slyke (2). Chlorides were determined according to field method No. 3 of Hayden (10), which merely indicates whether the chlorine content is above or below 0.14 per cent.

The numbers of bacteria were determined by the standard plate method,

using plain nutrient agar and also blood agar containing 5 per cent defibrinated horse blood and 1 per cent dextrose. X

EXPERIMENTAL

Table 1 presents data portraying typical conditions found in milk from normal udders and from udders showing sub-clinical and acute mastitis. This table is presented to show the relative value of each method and to show the great contrast that exists in the quality of milk from the various quarters of the same udder. The data presented here depict definite clinical results which would characterize a normal udder or a diseased udder. Even the acute or clinical mastitis presents a different picture from the sub-acute or chronic mastitis. This however is not exactly a true picture of the problem for actually there appears to be varying degrees of intensity from the most minute indication to the severe acute or clinical type in which the udder becomes inflamed, the milk flow stops almost entirely—a spongy, amber-colored, purulent mass is discharged instead—and the animal is in great discomfort. Then, too, no single determination can give a true picture of the case, for we find that some cows having sub-acute cases frequently develop the acute stage only to subside to the sub-acute again, whereas several animals which have been under observation for five years have never had an acute attack during this time and yet have maintained constantly a typical picture of sub-clinical mastitis. During the course of these studies several animals have developed acute mastitis which has disappeared entirely, but generally an udder which is once badly infected will continue to produce milk of high leucocyte and catalase content after the swelling has disappeared and the milk has returned to normal appearance.

Udall and Johnson (26) claim that a physical examination of the udder rates above any other single method. They point out that pathological changes in the udder are located more uniformly and certainly by this method because of the tissue change fibrosis—scar tissue which develops as the result of infection. They point out that bacteriological methods may fail because grossly diseased udders do not give off streptococci constantly. During the course of these studies the cows have been under constant veterinary supervision, and detailed physical examinations of the udders have been made. These examinations have demonstrated the presence of fibrosis and lack of uniformity in consistency of the quarters in more than half of the animals under observation; however, some of these animals are high-producing cows whose milk is entirely normal, according to the chemical and bacteriological methods used in the laboratory. These studies indicate that the physical examination of the udder possesses merit for locating tissue change due to disease, which probably would serve as a practical basis for segregating diseased cows. Chemical and bacteriological methods alone, however, can establish the actual quality of the milk produced; practical

TABLE 1
Table Showing Simultaneous Tests On Milk From Individual Quarters

COW NO.	QUARTER NO.	BACTERIA PER CC.		TYPE OF INFECTION	LEUCOCYTES PER CC.	CATALASE TEST CC OXYGEN	pH	CHLO- RIDE TEST	CURD TENSION (GRAMS)
		Plain agar	Blood agar						
89	1	150	100	Normal Udders	11,000	.75	6.6-	0	39
	2	150	300		11,000	1.00	6.6-	0	46
	3	50	200		22,000	1.50	6.6-	0	46
	4	200	500		22,000	2.00	6.6-	0	40
*4X	1	0	100	88,000	2.00	6.6+	++	22	
	2	0	0	88,000	2.00	6.6+	+	27	
	3	0	300	22,000	1.25	6.6-	0	22	
	4	800	900	44,000	3.00	6.6-	+	22	
170	1	50	0	22,000	1.50	6.5	0	102	
	2	50	200	44,000	1.00	6.5	0	87	
	3	100	100	88,000	1.00	6.5	0	113	
	4	550	0	22,000	1.50	6.5	0	93	
172	1	300	300	22,000	0.75	6.5	0	65	
	2	200	300	22,000	0.75	6.5	0	72	
	3	0	200	22,000	0.50	6.5	0	80	
	4	200	0	22,000	0.75	6.5	0	67	
165	1	0	0	22,000	0.50	6.5	0	55	
	2	0	0	44,000	0.25	6.5	0	50	
	3	0	0	22,000	0.25	6.5	0	72	
	4	100	100	44,000	0.75	6.5	0	55	
175	1	1,000	0	22,000	0.75	6.5	0	75	
	2	700	0	44,000	1.50	6.5	0	80	
	3	100	0	22,000	0.75	6.5	0	85	
	4	100	200	22,000	2.00	6.55	0	75	
1X	1	0	0	Acute Mastitis	176,000	8.50	6.7+	+++	20
	2	900	5,100		10,000,000	18.00	7.3	++	0
	3	200	100		22,000	2.50	6.9	+	18
	4	0	0		44,000	5.25	6.9	+	18
164	1	200	100	224,000	8.25	6.7	+	43	
	2	150	0	176,000	6.00	6.7	0	57	
	3	100	100	176,000	8.50	6.7	0	55	
	4	2,000	1,800	2,508,000	17.00	7.0	+++	0	
151	1	3,200	250,000	Diplo. & Strep.	858,000	6.25	6.7+	0	67
	2	100	600	Streptococcus	858,000	5.00	6.7-	0	67
	3	41,000	60,800	Streptococcus	7,480,000	14.50	6.7-	0	63
	4	700	2,100	Staphylococcus	17,600,000		7.0+	+++	0

*It is very probable that this cow had an extremely mild infection.

TABLE 1.—(Continued)
Table Showing Simultaneous Tests On Milk From Individual Quarters

COW NO	QUAR- TER NO.	BACTERIA PER CC.		TYPE OF INFECTION	LEUCOCYTES PER CC.	CATALASE TEST ON OXYGEN	PH	CHLO- RIDE TEST	CURD TENSION (GRAMS)
		Plain agar	Blood agar						
61	1	600	2,600	Acute Mastitis	2,068,000	11.00	7.0	+++	18
	2	150	1,000		2,024,000	9.75	7.0	++	18
	3	0	100		1,980,000	12.50	6.8	0	27
	4	0	0		572,000	7.00	6.8	0	23
98	1	0	1,100	Streptococcus	1,628,000	7.50	6.9-	+++	22
	2	150	0		572,000	5.50	6.6-	+	28
	3	100	200		924,000	4.75	6.7	+	33
	4	0	10,500		1,012,000	18.00	6.9	++	12
150	1	0	0	Sub-clinical Mastitis	2,200,000	10.00	6.7	0	46
	2	100	0		176,000	1.00	6.7	0	75
	3	4,500	32,000		9,680,000	17.50	6.9	0	30
	4	0	0		22,000	.50	6.7	0	85
78	1	0	450,000	Streptococcus	24,200,000		6.2	+++	12
	2	100	700		880,000	14.25	6.9	0	16
	3	200	800		5,280,000	18.00	6.8	0	12
	4	100	900		2,420,000	14.00	6.9	0	9
52	1	200	1,100	Staphylococcus	308,000	16.25	6.7	0	41
	2	100	0		616,000	8.25	6.7+	0	34
	3	0	100		132,000	18.00	6.9	+	32
	4	0	1,200		10,000,000	18.00	7.0	0	12
160	1	50	100	Streptococcus	22,000	2.0	6.8	0	49
	2	0	0		22,000	1.25	6.6	0	63
	3	300	700		22,000	1.0	6.5	0	72
	4	50	0		858,000	11.0	6.6	0	37
24X	1	100	100	Streptococcus	886,000	3.75	6.5	+	47
	2	1,000	1,000		1,056,000	9.5	6.5	+++	27
	3	300	100		704,000	4.0	6.5	+	50
	4	0	11,600		396,000	6.0	6.5	+++	27
88	1	0	3,400	Streptococcus	88,000	8.0	6.8	0	18
	2	1,200	4,200		1,000,000	12.25	6.8	0	15
	3	1,300	5,400		572,000	7.00	6.7+	0	25
	4	350	800		1,760,000	14.50	6.9	0	15
167	1	200	5,900	Streptococcus	2,200,000	9.00	6.9-	0	15
	2	100	5,100		660,000	9.00	6.8+	0	15
	3	100	400		748,000	12.50	6.8+	0	14
	4	300	700		1,012,000	12.00	6.9+	0	15
18X	1	0	0	Streptococcus	44,000	0.50	6.6	0	22
	2	250	400		22,000	1.50	6.6-	0	23
	3	400	100		22,000	0.25	6.6-	0	29
	4	200	200		880,000	9.50	6.6+	0	23

methods and standards can be developed which would detect market milk from diseased udders. Thus they serve the double purpose of insuring the public a milk supply free from this objectionable condition and furnishing an incentive for dairyman to use such sanitary methods as are necessary for the control of the disease.

LABORATORY METHODS

The efficiency of the several tests used must be established by determining the extent to which the results are in agreement. Most of the cases reported here have been under observation from three to five years, and the data presented can be regarded as typical of the voluminous observations and determinations that have been made.

DIRECT MICROSCOPIC EXAMINATIONS

The authors agree with Hucker *et al.* (12) that direct microscopic examination of freshly drawn milk for streptococci has limited significance in indicating infected udders of the sub-clinical type, for only rarely does milk as drawn from the udder carry sufficiently large numbers of streptococci to be detected microscopically without at the same time being abnormal in appearance. Hucker *et al.* (12) were able to detect streptococci in milk which harbored few organisms by incubating the samples at 37 degrees Centigrade over night before making the final examination. The authors have employed this method with carefully drawn samples and found it effective for bringing about an enrichment of the organisms, especially upon the addition of 1 per cent dextrose. The mere presence, however, of long chain streptococci in incubated samples cannot be taken as positive evidence of udder infection. It is of value only when correlated with other findings.

PLAIN NUTRIENT AGAR AND BLOOD AGAR COUNTS

The counts on plain agar and blood agar do not always agree, but in general it may be said that the blood agar counts are much higher than those on plain agar. Plain agar is poorly suited for the detection of streptococcic mastitis because the colonies formed are usually so small that they are not detected with the naked eye. During the course of this work it was found advantageous to add 1 per cent dextrose to the blood agar because it caused the colonies to grow large enough to be counted readily. The principal objection to the dextrose is that it interferes with the hemolytic characteristics of the organisms. It will be observed that the agar count by either of these methods is not a satisfactory index of udder infection because occasionally no colonies are formed when it is very apparent from other tests that mastitis does exist. This probably is because the diseased condition was not active at that time. Therefore, it would be impossible to

say that any given number of bacteria are permissible and normal, and that more than this number means that the quarter from which the milk is drawn is diseased.

SIGNIFICANCE OF THE NUMBER OF CELLS

It is impossible to reconcile the divergent views expressed concerning the significance of the cell content of milk. These data were secured by taking a standard loopful of milk and spreading it over an area of one square centimeter. This film is stained by the rapid (one solution) method of Newman (21) and counted under the oil immersion objective of the microscope. The experience of this laboratory has developed reliance in this method. Though millions of leucocytes are found in the clinical or acute mastitis, they are also quite numerous in sub-clinical cases. The results of this paper are in agreement with the previous publication (7), which pointed out that more than 100,000 leucocytes per cubic centimeter is a good indicator of udder infection.

CATALASE TEST

This test has been run according to the method described by Orla-Jensen, in which a 20 cc. capacity test-tube is filled with 15 cubic centimeters of milk and sufficient 3 per cent peroxide to fill the tube. A rubber stopper containing a piece of bent glass tubing is inserted and the tube is inverted. This simple test has proved very satisfactory. An examination of the data shows a very close agreement to exist between this test and the cell or leucocyte count. Orla-Jensen claimed that milk from healthy cows would not yield more than 2.5 cubic centimeters of oxygen according to his technic. The data presented here conforms well to this standard.

TABLE 2
Relation of Serum and Dilution to Catalase Test and Curd Tension

SAMPLE TESTED	CURD TENSION (GRAMS)	CATALASE TEST (CC. OF OXYGEN)	TIME OF REACTION
Normal milk	111	0.5	6 hrs.
Milk + 5% water	116		
Milk + 5% serum	90	12.0	6 hrs.
Milk + 10% water	100		
Milk + 10% serum	88	16.0	6 hrs.
Milk + 15% water	85		
Milk + 15% serum	65	20.0*	45 min.
Milk + 20% water	80		
Milk + 20% serum	64	20.0*	17 min.
Milk + 50% water	40		
Milk + 50% serum	7	20.0*	4.5 min.
Serum (fresh)		20.0*	1.5 min.

*Complete displacement of liquid.

Table 2 shows the close relationship which exists between the amount of serum which gets into the milk and the catalase test. The serum used in this test was fresh, clear, straw-colored serum, quite free from blood cells. Blood cells contain such a great catalase content that when one drop of whole blood is added to 15 cubic centimeters of water or milk, and peroxide is added, complete displacement of the liquid by oxygen will take place. These data indicate the great catalase content of blood serum. The simplicity and reliability of the catalase test recommend it as a routine test for locating active infection in the cow's udder.

H-ION CONCENTRATION

Both colorimetric and electrometric methods have been employed for the determination of the H-ion concentration. The data reported here were obtained by using the quinhydrone electrode. The H-ion concentration appears to be poorly suited to the detection of sub-clinical mastitis. Occasionally, as in the case of cow No. 78 (Table 1), the milk from a diseased udder actually is more acid than normal. Generally, however, the H-ion concentration is less in milk from disease udders. In sub-clinical mastitis this difference frequently is so small as to be impossible to interpret. In acute mastitis the milk generally is alkaline; however, it is usually so abnormal in appearance under these conditions that there is no necessity for clinical identification. The H-ion concentration has limited merit as a clinical method for the detection of mastitis because the variation in reaction between milk from normal udders and udders showing sub-clinical mastitis is neither consistent nor wide enough in range to permit accurate interpretation.

SIGNIFICANCE OF EXCESSIVE CHLORINE CONTENT

The rapid test No. 3 reported by Hayden (10) was used during this investigation. This test is based on the principle that the presence of more than 0.14 per cent chlorine in the milk indicates that blood serum is entering the milk. This test is simple and easily interpreted. An examination of Table 1 shows that it is generally positive in acute mastitis, and questionable in cases of sub-clinical mastitis. Differences in the normal salt concentration of the milk from individual cows and also the actual amount of salt being consumed by the cow, as well as the degree of the disease, operate to mask the value of this test.

CURD TENSION

The curd tension was measured in grams according to the method of Hill (11). The accuracy of the spring balance used in these tests was limited because it was marked off in 5-gram divisions. However, the duplicate readings usually checked within 5 grams, which was adequate for the purpose of this experiment. It will be observed that normally there is a great

difference in the curd tension of milk from different cows. There is also a small normal variation in the curd tension of milk from the different quarters of the same udder. However, an acute case of mastitis often completely destroys the coagulating power of the milk. Sub-clinical mastitis similarly results in the lowering of the curd tension. Table 2 shows the comparative effect of added blood serum and water upon the curd tension of milk. These data reveal that blood serum has a much greater effect in reducing the curd tension than the same degree of dilution by water. A study of these data suggests the advisability of always running a catalase test on any sample of milk which is being tested for curd tension with the view of using the milk for infant feeding.

TYPE OF ORGANISMS PRESENT

Microscopic examination of thousands of stained preparations made from blood agar plates and films of fresh milk and incubated milk shows that nearly all cases of mastitis encountered during these studies were streptococcic. Occasionally a staphylococcus is found, and in one case one of the authors (6) encountered *Pseudomonas aeruginosa* as the causative agent.

CLINICAL TESTS

Chemical methods regularly employed for the detection of pus in urine have been tried on milk which was known to originate from cases of sub-clinical mastitis. Vitali's, Donne's, the benzidin and phenolphthalein tests have all been uniformly negative. It is possible that future experiments based on the detection of nucleic acids or their derivatives may be employed successfully to locate pyogenic infections in the cow's udder.

TESTS APPLIED TO MARKET MILK SAMPLES

Table 3 shows the results of applying these tests to retail milk samples picked up by the dairy inspector in his regular tour of duty. The bacterial count here has little significance except to indicate the general sanitary quality of the milk. The significance of leucocytes in the milk from individual cows has been shown previously. The possible application of standards based on such results to the control of market milk can well be ascertained from the results in table 3. If 100,000 leucocytes per cubic centimeter were accepted as a limit, then only 20 of the 54 samples could be retailed. The catalase test shows a close relationship to the leucocyte content except in the case of pasteurized milk, where the catalase is evidently destroyed. The pH value of milk apparently has no significance when applied to retail milk samples. The increased pH values characteristic of milk from infected quarters is lost by dilution with normal milk. It is significant to observe that chlorine in excess of 0.14 per cent was en-

TABLE 3
Tests Made on Retail Milk Samples

DAIRY NO.	BACTERIA PER CC.		LEUCOCYTES PER CC.	CATALASE TEST CC OXYGEN	PH	CHLORIDE	CURD TENSION (GMS.)
	Plain agar	Blood agar					
1	9,000	10,000	414,000	5.5	6.5	+++	20
2	18,800	39,200	1,380,000	1.0	6.5	-	38
3	23,000	20,800	3,542,000	8.0	6.5	-	50
4	38,000	23,000	1,104,000	4.0	6.5	-	37
5	11,400	9,300	46,000	2.25	6.5	-	100
6	48,200	62,000	92,000	1.75	6.5	-	35
7	9,000	24,000	92,000	3.75	6.5	-	65
8	3,800	5,300	92,000	2.25	6.5	-	67
9*	2,700	300	920,000	0.25	6.5	-	36
10*	12,000	15,800	184,000	0.25	6.5	-	39
11	131,000	43,000	690,000	6.75	6.5	+	101
12	1,000	4,400	23,000	5.0	6.5	-	43
13	7,200	6,500	552,000	6.25	6.5	++	65
14	13,400	5,800	23,000	0.5	6.5	++	48
15	6,000	16,000	230,000	3.5	6.5	+	46
16	21,000	19,800	276,000	3.75	6.5	-	45
17	1,800	3,000	230,000	3.75	6.5	-	37
18	32,000	31,000	138,000	4.00	6.5	-	47
19	12,950	15,000	138,000	3.00	6.5	-	47
20	43,400	35,000	92,000	3.5	6.55	-	50
21	35,000	214,000	484,000	4.0	6.5	-	56
22	27,000	41,600	176,000	2.5	6.5	-	55
23	6,050	7,100	308,000	3.0	6.5	-	50
24	35,500	120,000	2,860,000	8.75	6.5	-	63
25	4,400	3,800	484,000	4.0	6.5	-	44
26	6,450	5,200	176,000	3.25	6.5	-	61
27	4,450	5,400	132,000	3.50	6.5	+	50
28	1,750	1,800	88,000	2.75	6.5	-	82
29	1,500	2,400	88,000	2.50	6.5	-	52
30	2,350	5,900	836,000	4.50	6.5	-	44
31	1,250	1,800	308,000	4.50	6.5	-	40
32	13,500	18,000	132,000	2.0	6.5	-	45
33*	12,000	33,600	88,000	0.25	6.5	-	58
34*	850	20,400	484,000	0.25	6.5	-	40
35*	500	700	44,000	0	6.6	-	49
36	12,600	5,600	132,000	2.00	6.6	-	80
37	5,650	12,800	220,000	2.50	6.5	-	55
38	5,100		934,000	3.5	6.5	-	91.0
39	7,100		46,700	1.5	6.5	-	57.5
40	8,300		934,000	4.0	6.5	-	37.0
41	2,300		186,000	4.5	6.5	+	50.0
42	2,700		46,700	1.25	6.5	-	60.0
43	73,000		46,700	1.5	6.5	-	57.0
44	42,000		93,400	3.75	6.5	-	37.5
45	4,100		93,400	1.5	6.5	-	30.0
46	16,400		93,400	2.0	6.5	-	35.0
47	27,000		93,400	1.5	6.5	-	25.0
48	14,800		1,961,000	4.75	6.5	-	47.5
49*	400		93,400	-	6.5	-	37.5
50	21,400		46,700	2.30	6.5	-	45.0
51	18,400		130,000	4.25	6.5	+	45.0
52	132,000		1,670,000	5.25	6.5	-	18.0
53	3,900		934,000	7.50	6.5	-	45.0
54*	2,700		93,400	-	6.5	-	37.5

*Pasteurized milk.

countered in milk which most certainly came from badly infected herds; however, it must also be observed that many samples showing excessive leucocyte and catalase content did not contain an excess of chlorine. Curd tensions vary over a wide range. Some samples possessing high leucocyte and catalase content possess high curd tensions, whereas other samples may possess low leucocyte and catalase content along with a low curd tension. These data again indicate the desirability of determining the presence of udder infection simultaneously with the curd tension.

SUMMARY

1. Acute mastitis in cows usually is easily recognizable. The affected quarters are intensely inflamed and the cow suffers much discomfort. The milk from these quarters is characterized by producing abundant colonies on dextrose blood agar though the count on plain agar may appear normal. The milk usually contains clots and its consistency is watery—in extreme cases the solids completely separate into a spongy mass which floats in an amber-colored serum. Milk from these quarters contains millions of leucocytes per cubic centimeter; the catalase content is extremely high, usually producing 10 cubic centimeters or more of gas according to the technic here used; the hydrogen-ion concentration decreases until the milk is neutral or slightly alkaline. However, isolated cases are found where the H-ion concentration actually increases; the milk usually contains chlorine in excess of 0.14 per cent, and the curd tension according to Hill's method is almost entirely destroyed.

2. Chronic or sub-clinical mastitis is the most common form. This type of infection is commonly so mild that it passes without recognition. The udder appears superficially normal; flakes or clots may appear occasionally but the milk usually appears normal. This condition is very common in dairy herds. The laboratory detection of this milk is best accomplished by examining the milk from individual quarters of the udder. In this type of mastitis the bacterial count often appears normal on plain agar; dextrose blood agar, however, usually reveals an abnormally high count. The leucocyte count is in excess of 100,000 per cubic centimeter, and the catalase test usually produces 2.5 cubic centimeters or more of gas, according to the technic here used; the H-ion concentration is usually only slightly reduced; the chlorine content of the milk is commonly normal; and the curd tension according to Hill's method is usually reduced.

3. The addition of clear fresh blood serum from blood cells to milk increases the catalase content markedly. The addition of serum also causes a decrease in the curd tension. Blood serum has a much greater effect in reducing the curd tension than the same degree of dilution by water.

4. Chemical tests regularly employed for the detection of pus in urine

have been negative when applied to milk known to originate from cases of sub-clinical mastitis.

5. The physical examination of the udder possesses merit for locating tissue change due to disease; however, chemical and bacteriological methods alone can actually establish the quality of milk which is produced.

6. Retail milk samples when subjected to these tests showed that 33 of the 54 samples tested contained catalase in sufficient quantities to produce 2.5 cubic centimeters or more of gas and 34 of the 54 samples contained more than 100,000 leucocytes per cubic centimeter. The discrepancy between the number of samples showing excessive leucocytes and catalase is due to the pasteurized samples in which the catalase has been destroyed. The H-ion concentration was normal in practically all samples. Only 8 of the 54 samples contained chlorine in excess of 0.14 per cent. The curd tension of herd samples apparently had no relation to the number of leucocytes or to the catalase test.

7. According to this study it appears safe to conclude the leucocytes in excess of 100,000 per cubic centimeter and catalase in sufficient quantity to produce 2.5 cubic centimeters of oxygen or more, according to the method reported by Orla-Jensen, are reliable indices of udder infection.

CONCLUSION

Of all laboratory methods employed in these studies for the detection of sub-clinical mastitis, the leucocyte and catalase content of milk serve as the most reliable indicators.

REFERENCES

- (1) BAKER, J. C., AND BREED, R. S. The reaction of milk in relation to the presence of blood cells and of specific bacterial infections of the udder. New York Agr. Exp. Station Tech. Bul. 80, pp. 239-255. 1920.
- (2) BAKER, J. C., AND VAN SLYKE, L. L. A method for the preliminary detection of abnormal milk. New York Agr. Exp. Station Tech. Bul. 71, pp. 1-14. 1919.
- (3) BREED, ROBERT S. Cells in milk derived from the udder. New York Agr. Exp. Station Bul. 380, pp. 139-200. 1914.
- (4) BREED, ROBERT S. The sanitary significance of leucocytes in milk. New York Agr. Exp. Station Bul. 568, pp. 1-14. 1929.
- (5) BREED, R. S., AND BREW, J. D. The control of bacteria in market milk by direct microscopic examination. New York Agr. Exp. Station Bul. 443, pp. 717-746. 1917.
- (6) CHERRINGTON, V. A., AND GILDOW, E. M. Bovine mastitis caused by *Pseudomonas aeruginosa*. Jour. A. V. M. A. 79 (N. S. 32, No. 6): 803-808. 1931.
- (7) CHERRINGTON, V. A., HANSEN, H. C., AND HALVERSEN, W. V. The leucocyte content of milk as correlated with bacterial count and hydrogen-ion concentration for the detection of mastitis. JOUR. DAIRY SCIENCE 16: 59-67. 1933.
- (8) HAMMER, B. W., AND BAILEY, D. E. A rapid volumetric method for approximate estimation of chlorine in milk. Iowa Agr. Exp. Sta. Research Bull. 41, pp. 337-348. 1917.

- (9) HASTINGS, E. G., AND HOFFMAN, C. Bacterial content of milk of individual animals. Wis. Agr. Exp. Sta. Bull. 6 (Research), pp. 189-196. 1909.
- (10) HAYDEN, C. E. Field tests for chlorine in milk for the detection of mastitis. Cornell Vet. 22: 277-280. 1932.
- (11) HILL, R. L. Soft-curd milk. Utah Agr. Exp. Station Bul. 227, pp. 1-23. 1931.
- (12) HUCKER, G. J., TRUDELL, FERDINAND, AND JENNINGS, WILLIAM S. The incidence and detection of sub-clinical streptococcus mastitis. New York Agr. Exp. Station Tech. Bull. 199, pp. 1-52. 1932.
- (13) HUCKER, G. J. Relationships of leucocytes and streptococci to fibrosa of the udder. Am. Jour. Pub. Health, 23: 237-245. 1933.
- (14) HUCKER, G. J. The laboratory detection of bovine mastitis. New York Agr. Exp. Station Bul. 626, pp. 1-24. 1933.
- (15) KASTLE, J. H. Chemical tests for blood. Hygienic Laboratory Bul. 51, pp. 5-59. 1909.
- (16) MANDEL, J. A., AND LEVINE, P. A. Glucothionic acid in leucocytes. Biochem. Z. 4: 78-79. 1907.
- (17) MATHEWS, ALBERT P. Physiological chemistry, 3rd edition. William Wood Co., New York. 1924.
- (18) MORGULIS, SERGIUS, BEBER, M., AND ROBIN, I. Studies on the effect of temperature on the catalase reaction. Jour. Biol. Chem. 68: 521-563. 1926.
- (19) MORGULIS, SERGIUS, AND BEBER, M. Studies on the effect of temperature on the catalase reaction. Jour. Biol. Chem. 77: 115-126. 1928.
- (20) MORGULIS, SERGIUS. Studies on the inactivation of catalase. Jour. Biol. Chem. 86: 75-85. 1930.
- (21) NEWMAN, R. W. A one-solution technique for the direct microscopic method of counting bacteria in milk. Monthly Bulletin, California State Dept. of Agriculture 16: 1-7. 1927.
- (22) ORLA-JENSEN. Dairy bacteriology. pp. 160-161. P. Blakiston's Son & Co., Philadelphia. 1921.
- (23) PRESCOTT, S. C., AND BREED, R. S. The determination of the number of body cells in milk by a direct method. Jour. Inf. Dis. 7: 632-640. 1910.
- (24) ROSELL, J. M. Streptococcic Mastitis. Cornell Vet. 21: 80-85. 1931.
- (25) TWEED, R. L. The relation of high cellular counts to *Bacterium abortus* infection of the udder. Mich. Agr. Exp. Station Tech. Bul. 61, pp. 1-28. 1923.
- (26) UDALL, D. H., AND JOHNSON, S. D. The diagnosis and control of mastitis. Cornell Vet., 21: 190-206. 1931.
- (27) VON FELLEBERG, T. Determination of purine bases in foods. Mitt. Lebensm. Hyg. 9: 73-84. 1918. (Als. Chem. Abstracts 12, pt. 2, p. 1798. 1918.)
- (28) WELLS, H. GIDEON. Chemical Pathology, Fifth Edition, pp. 298. W. B. Saunders Co., Philadelphia. 1925.
- (29) WELLS, H. G. The relation of autolysis to the histological changes occurring in necrotic areas. Jour. Med. Res. 15: 149-165. 1906.

RATE OF PASSAGE OF INERT MATERIALS THROUGH THE DIGESTIVE TRACT OF THE BOVINE*

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A knowledge of the rate of passage of inert materials through the digestive tract is of importance in digestibility studies. This information is necessary to determine how long feeds may exert an influence where rations are changed from one feeding régime to another in order that proper interpretation may be given to the results. The development of a method for determining this rate of passage also offers a means for investigating the comparative costive or laxative effect of feeds.

Hoelzel (1) studied the rate of passage of inert materials through the digestive tract of rabbits, guinea-pigs, dogs, cats, albino rats, white mice, pigeons, one monkey, one hen, and one man. The test materials included rubber, cotton thread (knots), seeds, glass beads, pieces of aluminum, steel, silver, and gold. The rate of passage of these inert materials was found to be more or less proportional to the specific gravity of the test materials, the heavier materials passing more slowly than the lighter ones. The rate of passage also varied considerably in the different species and individuals.

Burnett (2) used 50 cc. of French millet seeds in studies with man, noting the number of hours five or more seeds were first and last seen on the surface of the naturally dejected feces. He considered 14-85 hours a rapid rate and 62-134 hours a normal rate.

Alvarez (3) used glass beads in determining the rate of passage of food residues through man and found on an average 75 per cent of the beads were passed in 96 hours.

Cugnini (4), cited by Burnett (2), placed powdered Brazil nuts in the food of horses in 33 tests. The first appearance of the marker varied between 15 and 24 hours and was last seen from 74 to 195 hours.

Reed, Huffman and Addington (5) obtained the rate of passage of Sudan III fed to four heifers. The first and last appearance of the dye was determined from the color of the filtered ether extract of the feces. The first appearance was from 12 hours and 45 minutes to 16 hours. The last appearance was from 47 to 51 hours.

Fish (6) fed Sudan III in butter to a cow and found that the dye first appeared in the feces 16 to 17 hours after feeding and the last appeared from 48 to 92 hours.

Ewing and Smith (7) fed rubber discs to steers as markers. Some of

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the discs were passed in from 12 to 60 hours and some were not passed at all. These investigators later adopted the slaughter method.

Mitchell, Hamilton and Kick (8) suggest, after feeding iron oxide to steers, that the rate of feeding may be an important factor in determining the time of passage of food through the digestive tract. In other words, the food consumed clears the tract of residues from preceding meals in proportion to its mass.

The reviewed literature reveals very few systematic studies of the rate of passage of inert materials through the digestive tract of the bovine. It was with this point in view that the experiments reported in this paper were carried out.

EXPERIMENTAL

In order to measure the rate of passage of inert materials through the digestive tract it is necessary to feed some substance which can be recovered from the feces. It is necessary to feed something which the animal does not digest nor absorb and which does not affect the digestive system. Furthermore, if solid materials are fed, consideration must be taken of the fact that the bovine regurgitates and remasticates a considerable portion of the food consumed. This fact eliminates the use of glass beads, seeds, etc. Iron oxide and rubber discs were used in this investigation.

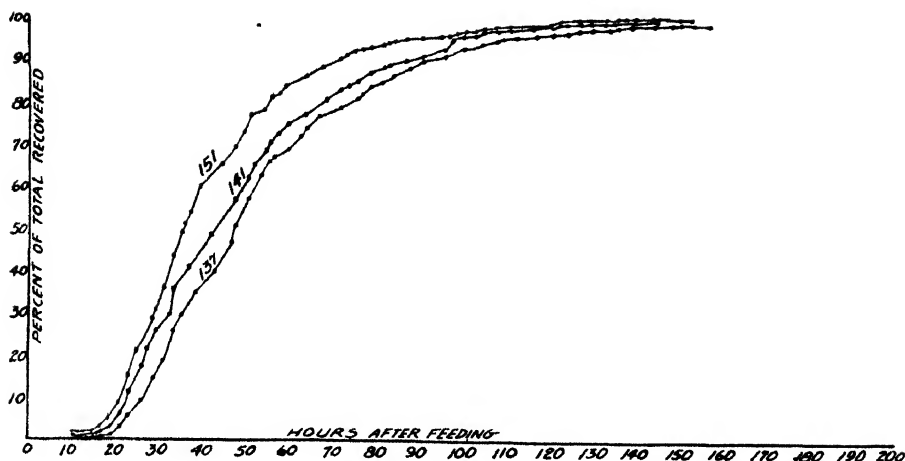
In all of the graphs in this report the total amount of the test materials recovered at the end of the experiment was called 100 per cent regardless of the amount fed. Any point on the graph represents the percentage of the total test material recovered which had been excreted at that particular time. In other words, in Graph I, 40 per cent of the total iron recovered had been accounted for after 42 hours for animal 137. In this manner the curves representing the rate of excretion of each animal can be compared with each other on a similar basis.

EXPERIMENT I

Iron Oxide

One hundred grams of the iron oxide (technical grade 83.1% Fe_2O_3) sufficiently fine to pass through a 100 mesh sieve was fed with the feed of silage to each of three healthy pure bred Holstein cows on the morning the experiment was begun. Constant watch was then kept for 215 hours. Each passage of feces was collected and the time of defecation noted. Each passage was weighed and the iron content of each sample determined by the method of Pincussen and Roman (9). The cows were exercised from 20 to 30 minutes each day during the experiment.

Complete data obtained from each animal were omitted due to the large amount of space required to submit them. Table I gives information relative to each cow and a summary of the results. The rate of excretion of the iron oxide is shown in Graph I.



GRAPH I. SHOWING RATE OF EXCRETION IN HOURS OF IRON OXIDE AS PERCENTAGE OF THE TOTAL RECOVERED FOR EXPERIMENT I.

The results show that the first iron excreted appeared in from 9 hours and 55 minutes to 13 hours and 20 minutes after feeding; that the high point of excretion was 33 hours and 10 minutes for all three animals; that the end points of excretion varied from 143 hours and 10 minutes to 156 hours and 5 minutes. The high point was considered that point at which the greatest amount of test material was excreted per kilo of feces. It was somewhat difficult to tell exactly the end point of excretion since the percentage of iron in the feces may come down to the basal percentage and then rise again slightly for a few passages. The point at which the percentage of iron in the feces did not rise again above the basal value was considered the end point.

Only 82.4 to 88.7 per cent of the iron oxide fed was accounted for. The method of analysis was checked for its accuracy without disclosing any discrepancy sufficiently great to account for the loss.

EXPERIMENT II

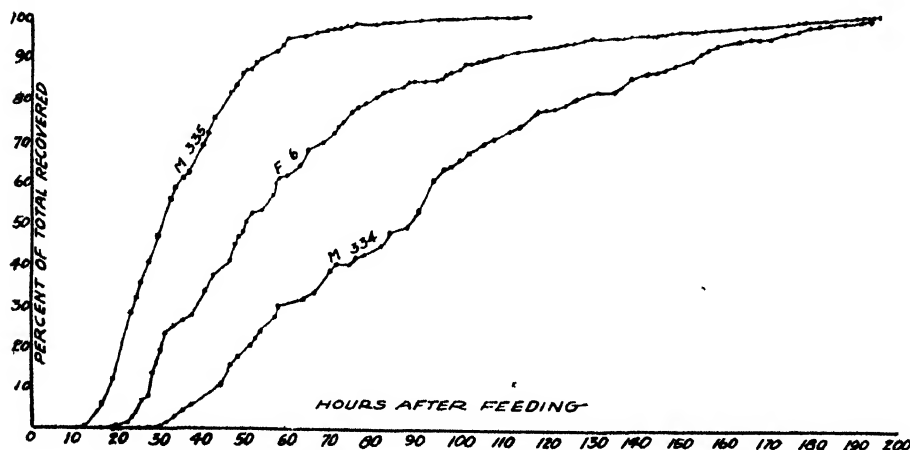
Rubber Rings

Rubber rings were cut about 1.5 to 2.0 millimeters in thickness from heavy walled white rubber tubing one-half inch in diameter with a wall one-eighth inch in thickness.

Three healthy grade Holstein cows were fed 1,000 rubber rings mixed in the silage portion of the ration. Those remaining in the manger after the silage had been consumed were mixed with a little grain so that all of the rings were finally consumed by the animals. One thousand rings occupied about one quart in volume. Constant watch was then kept of the animals. Records were kept of the daily food and water consumption.

The water consumption was determined by means of water meters attached to the drinking cups. The animals were kept on the experiment for 200 hours. They were exercised from 20 to 30 minutes each day.

Each passage of feces was weighed, the time noted, and the hardness or softness determined by mechanical means. The consistency of feces was determined in order to ascertain whether or not the animals remained in about the same physiological condition throughout the experiment insofar as the digestive tract was concerned. Each passage of feces was then washed on a one-eighth mesh screen with a stream of water from a hose to recover the rings. Data pertaining to the animals and a summary of the results obtained are shown in table 1. The rate of excretion of the rings is shown in Graph II.



GRAPH II. SHOWING RATE OF EXCRETION OF RUBBER RINGS AS PERCENTAGE OF THE TOTAL RECOVERED FOR EXPERIMENT II.

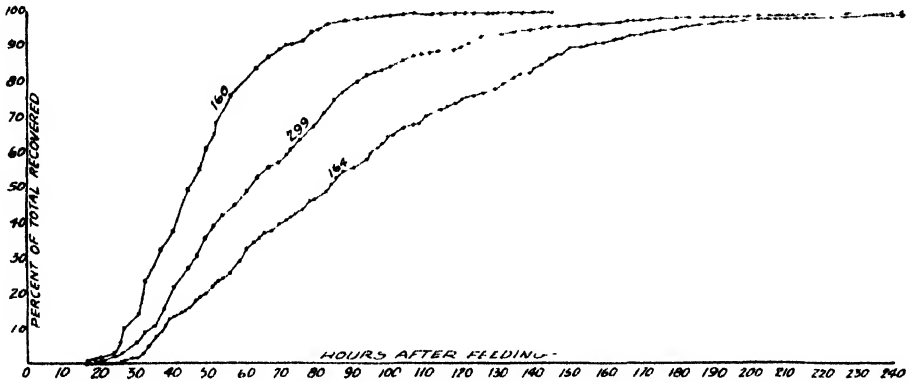
Not all of the rubber rings were recovered except in the case of animal M 335. The first appearance in the feces was from 10 hours and 45 minutes to 18 hours and 7 minutes. The high point of excretion was from 23 hours and 12 minutes to 33 hours and 15 minutes. The end point of excretion could be determined for only one animal, M 335, which excreted the last ring at 114 hours and 35 minutes. The rings obtained from animals M 334 and F 6 along toward the latter part of the experiment were considerably cut up. It was necessary to piece the parts together in counting the rings. This of course introduced some error. The consistency of the feces of the animals varied but little from day to day during the progress of the experiment.

EXPERIMENT III

Rubber Rings

The second experiment using rubber rings was practically a duplicate of the first one except that no silage was included in the feeds. The rings

were fed in the grain mixture. The animals used were three healthy pure-bred cows, kept under constant watch for 215 hours after the rings were fed. Information and data concerning the animals of this experiment are shown in table 1. The rate of excretion of the rings is shown in Graph III.



GRAPH III. SHOWING RATE OF EXCRETION OF RUBBER RINGS AS PERCENTAGE OF THE TOTAL RECOVERED FOR EXPERIMENT III.

All of the rings were not collected from two of the animals. More rings were supposedly recovered from animal 164 than were fed. This discrepancy is due to the error in estimating the number of rings from the pieces or fragments.

The feces of these animals were collected for 72 hours after the 215 hours' period. Constant watch was not kept during this period but the feces were washed about every 12 hours to obtain the rings. The first appearance of the rings was from 17 hours to 19 hours and 30 minutes. The high point of excretion was from 37 hours and 15 minutes to 60 hours and 30 minutes. The end point of excretion was 141 hours and 52 minutes for animal 164, although one-fourth of a ring was excreted between 226 and 241 hours. It is difficult to set an end point for the other two animals since 100 per cent of the rings was not recovered. However, cow 299 excreted only four and two-thirds rings between 193 hours and 52 minutes and 241 hours, after which none were excreted up to 287 hours when the experiment ended. Animal 160 excreted only three rings between 214 hours and 30 minutes and 287 hours, although only 83 per cent of the rings had been accounted for. The curves showing the rate of excretion of the rubber rings show considerable variation. Animal 160 was exceedingly slow.

The consistency of the feces varied but little during the progress of the experiment. The animals on this experiment which received no silage consumed considerably more water than the animals on the previous experiment which received silage.

DISCUSSION OF RESULTS

The use of iron oxide might be criticized because of its possible astringent effect although it is supposed to be practically insoluble in the digestive tract. Hoelzel (1) was inclined to believe that iron oxide acted as an astringent. In Experiment I of the present paper 400 rubber rings fed to the cows 48 hours after the iron oxide had been fed was excreted at almost the identical rate at which the iron had been excreted for each cow. The fact that not all the iron oxide fed was recovered is not surprising in view of the work of Hoelzel (1), who found that in animals with which he worked certain materials might be retained in parts of the digestive tract for considerable periods of time. Gallup (10), and Heller, Breedlove and Likely (11) were unable to recover 100 per cent of the iron oxide fed to rats when the Bergeim (12) method for studying digestibility was used. Furthermore, in this investigation not all the rubber rings were recovered, although a better percentage recovery was secured with rubber rings than iron, after a considerable period of collection which demonstrated that they had become lodged or settled in some part of the digestive tract. These results indicate that the Bergeim (12) method of investigating digestibility where iron oxide is used would not be accurate for the bovine.

It is interesting to note that animal 151, in which the iron oxide first appeared in the feces in 9 hours and 55 minutes after being fed, showed the fastest rate of excretion. Animal 141, in which the iron oxide appeared in 10 hours and 35 minutes, showed the medium rate, while animal 137, in which the iron oxide first appeared in 13 hours and 20 minutes, showed the slowest rate of excretion. In Experiment II, M 335, the animal which first started to excrete the rings and from which the most rings were recovered, reached the high point of excretion first and showed the fastest rate of excretion. The other two animals follow in a like manner in these respects. In Experiment III, the animal which first started to excrete the rings, also reached the high point of excretion first, and from which the most rings were recovered also showed the fastest rate of excretion. Animal 160 proved to be an exception in one respect in that she excreted the first ring in 17 hours which was the same as Animal 164, but showed the slowest rate of excretion. However, 160 did not excrete any more rings until 5 hours later when she excreted four, although she made two defecations in the mean time. By 30 hours she had excreted only 13 rings while animal 164 had excreted 104.

It appears from the results of this investigation that under comparable conditions those animals which first excrete the test material and reach the high point of excretion first, have the fastest rate of excretion. A study of the graphs indicates that the feeding of a test material and collection for 60 hours afterwards gives a comparable method for studying the rate of passage of inert materials through the digestive tract. It is difficult to measure

the complete lag, probably due to the fact that the test material becomes lodged or settles in some part of the digestive tract and may not be excreted for a considerable period of time.

These results indicate that feeds fed to bovine may exert some influence even up to 150 hours after feeding. They also show that in experiments with the bovine where the feeds are changed from one régime to another the lag must be taken in consideration in the interpretation of results.

The animals used in this investigation were on a medium plane of nutrition. It would be interesting to study the effect on the rate of passage of inert materials in animals on a high and a low plane of nutrition. It would likewise be interesting to study the rate of passage from the abomasum, excluding the rumen, reticulum, and omasum. However, due to the expense involved in keeping constant watch these points have not been investigated.

SUMMARY AND CONCLUSIONS

1. The rate of passage of iron oxide through the digestive tract was studied in three cows. The rate of passage of rubber rings was studied in six cows.

2. The iron oxide first appeared in the feces in from 9 hours and 55 minutes to 13 hours and 20 minutes. The rubber rings first appeared in from 10 hours and 45 minutes to 19 hours and 30 minutes.

3. The high point of excretion of the iron oxide was 33 hours and 10 minutes. The high point of excretion for the rubber rings was from 23 hours and 12 minutes to 60 hours and 30 minutes.

4. The lag varied from 114 hours and 35 minutes to 156 hours and 5 minutes for the iron oxide and from 141 hours and 52 minutes to approximately 215 hours for the rubber rings.

5. It appears that those animals under comparable conditions which first excrete the test material and first reach the high point of excretion show the fastest rate of excretion.

6. The feeding of a test material, collection and examination of the feces for 60 hours afterwards gives a comparable method for investigating the rate of passage of inert material.

REFERENCES

- (1) HOELZEL, FREDERICK. The rate of passage of inert materials through the digestive tract. *Amer. Jour. Physiol.* 92: 466-497. 1930.
- (2) BURNETT, F. L. Variations of the intestinal rate. *Jour. Amer. Med. Assoc.* 85: 1777-1781. 1925.
- (3) ALVAREZ, WALTER C. The mechanics of the digestive tract, p. 275-282. Paul B. Hoeber, Inc. New York. 1928.
- (4) CUGNINI, cited by BURNETT (2). *Esperienze sulla digestione nei cavalli. Clinica Veterinaria, Sezione Pratica Settimanale* 31: 436-440. 1908.

TABLE 1
Information relative to the animals used and a summary of the results obtained

ANIMAL	AGE	WEIGHT	AVERAGE DAILY							RESULTS			
			Grain Fed	Hay Fed	Corn Silage Fed	Water Consumption	Milk Production	Defecations	Feces	First Appearance	High Point	Last Appearance	Total Recovered
No.	gys.	lbs.	lbs.	lbs.	lbs.	lbs.	' '	No.	gms.	hrs. & min.	hrs. & min.	per cent	
Experiment I. 100 grams iron oxide fed													
151	8	1450	4	12.0*	24		0.0	9.6	23609	9:55	33:10	152:55	84.7
141	8½	1450	4	12.0*	24		0.0	9.0	23254	10:35	33:10	143:10	82.4
137	9	1600	4	12.0*	24		8.3	9.0	21518	13:20	33:10	156:05	88.7
Experiment II. 1,000 rubber rings fed													
M 334	5	1311	10	9.0**	30	47	15.4	9.1	19661	18:07	33:15		95.0
M 335	5	1390	11	10.0**	30	61	1.8	11.7	25596	10:45	23:12	114:35	99.9
F 6	3	1062	15	8.0**	20	79	40.7	12.6	22820	15:58	28:17		96.5
Experiment III. 1,000 rubber rings fed													
229	5	1319	6	18.5***	0	80	14.0	8.8	22877	19:30	40:35		90.8
164	3	1184	8	17.9***	0	82	19.7	8.4	21735	17:00	37:15	141:52	100.5
160	3	1138	10	17.5***	0	76.2	15.0	13.2	25754	17:00	60:30		83.0

* Coarsely cut mixed hay.

** Coarsely cut timothy.

*** Alfalfa.

- (5) REED, O. E., HUFFMAN, C. F., AND ADDINGTON, L. H. Cottonseed meal as a feed for dairy calves. *JOUE. DAIRY SCI.* 11: 488-515. 1930.
- (6) FISH, P. A. The rate of passage of material through the digestive tract. *Cornell Veterinarian* 13: 82-92. 1923.
- (7) EWING, P. V., AND SMITH, F. H. A study of the rate of passage of food residue through the steer and its influence on digestion coefficients. 1917.
- (8) MITCHELL, H. H., HAMILTON, T. S., AND KICK, C. H. Feeding rate determines speed of passage. *Illinois Station Report*, p. 117. 1928.
- (9) PINCUSSEN, L., AND ROMAN, W. Eine Methode zur Bestimmung von Ferri-Ionen, Ferro-Ionen un organisch gebundenum Eisen in biologischem material. *Biochem. Z.* 231: 54-58. 1931.
- (10) GALLUP, W. D. The digestibility of the proteins of some cottonseed products. *Jour. Biol. Chem.* 76: 43-53. 1931.
- (11) HELLER, V. G., BREEDLOVE, C. H., AND LIKELY, W. A comparison of the Bergeim and standard methods of determining coefficients of utilization with suggested modifications. *Jour. Biol. Chem.* 79: 275-282. 1928.
- (12) BERGEIM, O. Intestinal chemistry. IV. A method for the study of food utilization or digestibility. *Jour. Biol. Chem.* 70: 29-33. 1926.

STUDIES ON WHIPPING CREAM. II*

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In an earlier paper¹ under this title studies were presented on whipping cream in which the factors discussed were: the fat content of the cream, the time and temperature of aging, the reaction of the cream as measured in terms of titratable acidity, and the effect of added salts. In this paper further data on the effect of reaction are given together with data on the following factors:

1. Temperature of Cream Separation.
2. Cream Pasteurization.
 - a. Temperatures.
 - b. Cooling after Pasteurization.
3. Homogenization of the Cream.
 - a. Temperatures.
 - b. Pressures.

The properties of the whipped cream studied were the same as before, namely: whipping time, stiffness of the whipped cream, overrun, and drainage.

The apparatus and procedures were the same as described in the first paper¹ so a brief summary will be sufficient at this time. The cream whipping was performed in a room having a temperature range of 38 to 44° F. (3.3 to 6.6° C.). The turbine whipper was driven by a motor of sufficient power to maintain a constant speed. The load on the motor and its rate of increase were noted at regular intervals from the gear arrangement and scale as described previously.¹ The terms used in the accompanying tables have been defined. "Average torque increase per second" is the figure obtained by dividing the increase in torque as expressed in grams by the number of seconds that the cream was whipped. As is evident from the curves this value varies as the whipping progresses, but the average value is useful for purposes of comparison. For example, if one sample of cream whipped through a torque of 88 grams in 220 seconds and another through

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¹ Hugh L. Templeton and H. H. Sommer. Studies on whipping cream. *JOUR. DAIRY SCIENCE*, 16: 329-345. 1933.

a torque of 116 grams in 290 seconds, the "average torque increase per second" would be the same, namely 0.400.

The overrun was determined by weighing a definite volume of cream and an equal volume of the whipped cream, care being taken to prevent undue agitation of the whipped cream.

The data presented in this paper may be taken as typical of the results obtained in the study of each of the factors enumerated. Space limitations do not permit the presentation of the complete whipping record of each sample given in the tables; only the "average torque increase per second" is given. A few curves are included to illustrate typical whipping records and the corresponding samples in the tables are indicated. Similar curves were drawn for each of the samples in the tables and it was found that the "average torque increase per second" is a reliable basis for comparison.

The reaction of cream to be used for whipping has received considerable attention. Apparently it is quite common for practical dairymen to consider "ripening" of cream as a possible remedy for unsatisfactory whipping. Accordingly the effect of reaction was studied comparing normal, sweet cream with cream that had been acidified by the addition of acids. The acidity of the cream is given in terms of titratable acidity calculated as lactic acid rather than in terms of hydrogen-ion concentration, because the average dairy plant does not have the equipment needed for the latter determination. The titratable acidity of both the cream and the serum are given. The acidity of the serum was determined by titrating a 9 cc. portion of the drainage from the first whipping of the cream.

The results of this study on the effect of reaction are presented in table 1 and figure 1. The table is divided into sections according to the fat content of the cream used, the results given in each section having been obtained on different batches of cream. Section "A" of the table is a comparison of the effects of added lactic and citric acids. It will be noted that the whipping time decreases as the titratable acidity increases and that the addition of citric acid gives more pronounced effects than lactic acid. The cream containing the more acid was rather viscous and the whipped cream was rather soft, but it held the serum very tenaciously as shown by the length of time necessary for drainage and the smaller amounts of serum that drained from the cream.

Section "B" gives the data for successive increases in the amount of added lactic acid. The results show the decrease in whipping time with increasing acidity. The sudden drop in the value for the overrun was expected from the viscosity of the cream and the fact that the whipped cream did not whip up, but remained as a rather soggy mass in the bottom of the dish when the torque indicated the maximum stiffness attainable with apparatus. It was noted from time to time in the different experimental whips that the cream with the most noticeable increase in viscosity did not

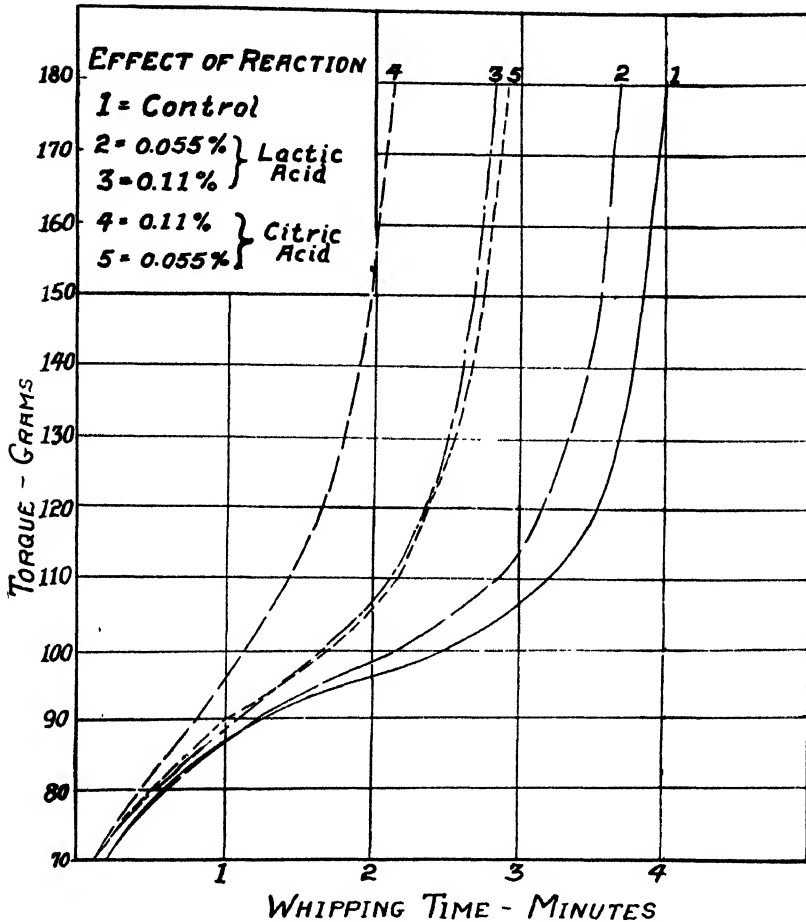


FIG. 1

whip as well as cream that was less viscous. It has also been noted in connection with the increase in acidity that there is a point where the overrun drops sharply. This usually occurs when the titratable acidity is about 0.30 per cent calculated as lactic acid. This high acid cream shows very little drainage and the fat content of the serum is very low.

Section "C" compares the effect of the addition of the acid before and after pasteurization. It is very interesting to note that with the increase in acidity above 0.20 per cent that the cream acidified before pasteurization whipped much more rapidly than that to which the acid was added after the cream had been cooled to 15° C. Below 0.20 per cent acid the creams whipped at about the same rate. The overrun was very uniform for the entire series. The values obtained in the study of the drainage are quite in agreement with those presented in the other sections of this table.

While the above results show that an increased acidity in general im-

TABLE 1
Effect of reaction on whipping quality

SAMPLE NO.	REAGENT ADDED		TITRATABLE ACID		WHIP TIME SEC.	AVERAGE TORQUE INC./SEC.	OVER-RUN %	DRAIN			
	Kind	%	Cream %	Serum %				Time sec.	Weight grams	Fat %	Fat loss
"A" 32% butterfat											
1	Control		0.11	0.13	239	0.551	165	763	42	4.43	1.87
2	Lactic Acid	0.055	0.15	0.19	222	0.588	169	476	37	6.10	2.27
3	"	0.11	0.19	0.21	170	0.778	173	1144	27	5.15	1.37
4	Citric Acid	0.11	0.26	0.26	128	1.018	162	1565	23	3.75	0.85
5	"	0.055	0.17	0.18	175	0.752	174	533	41	5.55	2.21
"B" 30% butterfat											
6	Control		0.11	0.13	346	0.373	166	351	57	3.65	2.10
7	Lactic Acid	0.011	0.13	0.14	394	0.327	160	369	56	3.63	2.01
8	"	0.033	0.14	0.16	336	0.370	166	454	57	4.05	2.31
9	"	0.055	0.16	0.19	324	0.389	170	415	56	5.05	2.82
10	"	0.088	0.19	0.22	294	0.422	171	415	47	5.23	2.43
11	"	0.11	0.21	0.22	259	0.493	171	579	31	4.40	1.37
12	"	0.22	0.31	0.32	186	0.711	141	1465	32	0.43	0.14
"C" 32% butterfat											
13	Control		0.11	0.14	172	0.707	162	487	45	5.45	2.43
14	Lactic Acid	0.055	0.15	0.20	154	0.813	165	617	40	5.28	2.11
15	"	0.055	0.16	0.20	156	0.795	168	393	51	5.43	2.72
16	"	0.11	0.21	0.22	133	0.939	168	1318	21	3.63	0.82
17	"	0.11	0.20	0.25	158	0.792	167	471	51	5.50	2.75
18	Citric Acid	0.055	0.17	0.21	157	0.778	165	496	44	5.55	2.41
19	"	0.055	0.19	0.21	149	0.813	165	334	49	6.03	2.93
20	"	0.11	0.23	0.26	104	1.199	162	1354	20	3.45	0.68
21	"	0.11	0.24	0.31	141	0.877	168	322	52	4.50	2.27

Acid added to samples Nos. 14, 16, 18, 20 before the cream was pasteurized; Acid added to samples Nos. 15, 17, 19, 21 after the cream had been pasteurized and cooled to 15° C.

proves the whipping of the cream, it does not follow that the beneficial effect of aging is due to an increase in acidity, since it is usually true that there is no measurable increase in acidity on aging. These results on the effect of reaction are in quite satisfactory agreement with the data presented by Babcock.² From work that will be presented later it would seem that one of the more essential features of aging is the solidification of the fat globules and the formation of clusters.

There are a number of factors involved in the production of satisfactory whipping cream that might be grouped under the general heading of plant procedures. The first of these to be presented is the temperature at which the cream is separated. The results of this study are given in table 2 and figure 2. The table has three sections and it should be noted that the cream used in the first two sections was from winter milk while the last series was

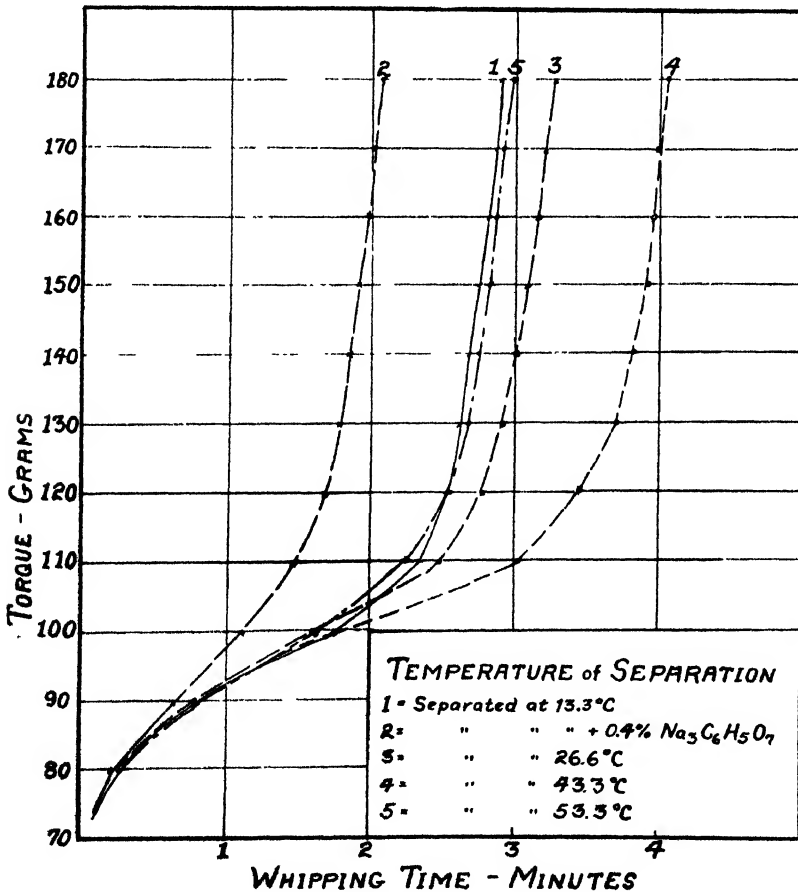


FIG. 2

² S. J. Babcock. The whipping quality of cream. U. S. Dept. Agr. Bulletin No. 1075. July 1922.

TABLE 2
Temperature of cream separation

CREAM			WHIP. TIME SEC.	AVERAGE TORQUE INC./SEC.	OVER- RUN %	DRAIN			
Sep. °F.	Temp. °C.	Fat %				Time sec.	Weight gms.	Fat %	Fat loss
I. Cream standardized to 32½ or 33% butterfat									
56	13.3	58	176	0.727	161	552	48	1.90	0.91
56*	13.3	58	124	1.016	154	373	54	3.00	1.61
80	26.6	55	196	0.641	161	499	47	4.48	2.12
80*	26.6	55	172	0.723	153	406	60	4.65	2.80
110	43.3	53	244	0.483	160	528	46	7.93	3.71
110*	43.3	53	163	0.768	151	527	55	6.48	3.59
128	53.3	49	177	0.711	160	415	51	10.35	5.24
128*	53.3	49	171	0.734	157	276	60	7.58	4.58
II. Cream standardized to 30% butterfat									
60	15.6	54	209	0.581	165	476	51	3.63	1.86
60†	15.6	54	180	0.645	157	421	54	2.60	1.40
85	29.4	56	196	0.605	161	587	50	4.50	2.24
85†	29.4	56	216	0.548	155	651	53	2.95	1.55
105	40.5	55	304	0.400	162	396	59	7.15	4.23
105†	40.5	55	168	0.724	161	436	61	9.55	5.90
126	52.2	51	440	0.266	170	441	59	6.15	3.60
126†	52.2	51	286	0.431	171	470	66	7.83	5.18
III. Cream standardized to 30-31% butterfat									
72	22.2	46	449	0.263	136	387	37	3.40	1.22
72†	22.2	46	396	0.284	122	324	40	2.40	0.95
100	37.8	36	623	0.097	130	94	62	9.20	6.22
100†	37.8	36	425	0.283	143	229	51	5.68	3.01
125	51.6	34	619	0.105	132	132	61	9.58	6.41
125†	51.6	34	494	0.200	135	195	55	6.30	3.49
150	65.5	31	424	0.289	150	371	44	7.25	3.25
150†	65.5	31	296	0.398	157	332	56	6.85	3.79

* 0.40% sodium citrate added to these samples before pasteurizing.

† 0.20% sodium citrate added to these samples before pasteurization.

‡ 0.40% sodium citrate added to these samples.

from milk produced in midsummer. This also accounts for the difference in temperature at which the first sample in each series was separated. All the creams were standardized with skimmilk collected at the same time as

the cream. The milk was heated in a tubular heater and the temperature taken of the milk as it entered the separator. The fat content of the creams separated at the different temperatures is given to show the decrease in fat with the increasing temperature. Sodium citrate in the amounts indicated was added to half of the samples in order to compare its effectiveness on the different creams. It should be noted that in all but one instance the addition of the citrate decreased the whipping time. In the first two series there are no significant differences in the overrun. It is evident that in order to minimize the fat losses in the serum it is wise to separate the cream at a low temperature. From the first and third series it is shown that the whipping time increases with the temperature and then decreases again. It is possible that if there had been a higher temperature used in the second series that the whipping time would have decreased at the higher temperature. No explanation can be offered at this time for the variations occurring in the third series especially in regard to the low overrun values.

The temperature at which the cream was pasteurized was found to have very little effect upon the whippability. As shown in table 3 and figure 3 there is a slight improvement in the whipping time and the length of time

TABLE 3
Temperature of pasteurization

PAST. TEM. °C.	WHIP. TIME SEC.	AVERAGE TORQUE INC./SEC.	OVER- RUN %	DRAIN			
				Time sec.	Weight gms.	Fat %	Fat loss
I. Cream containing 36% butterfat							
60-61.5	128	1.027	153	829	26	4.00	1.00
60-61.5*	141	0.898	138	616	30	2.95	0.87
65-66	130	1.013	152	600	23	7.50	1.67
65-66*	150	0.843	135	887	28	4.75	1.33
70-71	116	1.124	152	792	22	9.25	2.01
70-71*	135	0.950	139	509	28	6.35	1.61
II. Cream containing 29½% butterfat							
60-61.5	371	0.320	155	458	40	2.50	1.00
60-61.5*	319	0.397	153	553	40	2.13	0.84
65-66	375	0.335	156	567	38	4.35	1.68
65-66*	255	0.526	160	413	47	4.23	2.12
70-71	340	0.358	156	610	40	5.00	1.97
70-71*	247	0.513	159	454	46	4.63	2.08

* 0.40% sodium citrate added to these samples.

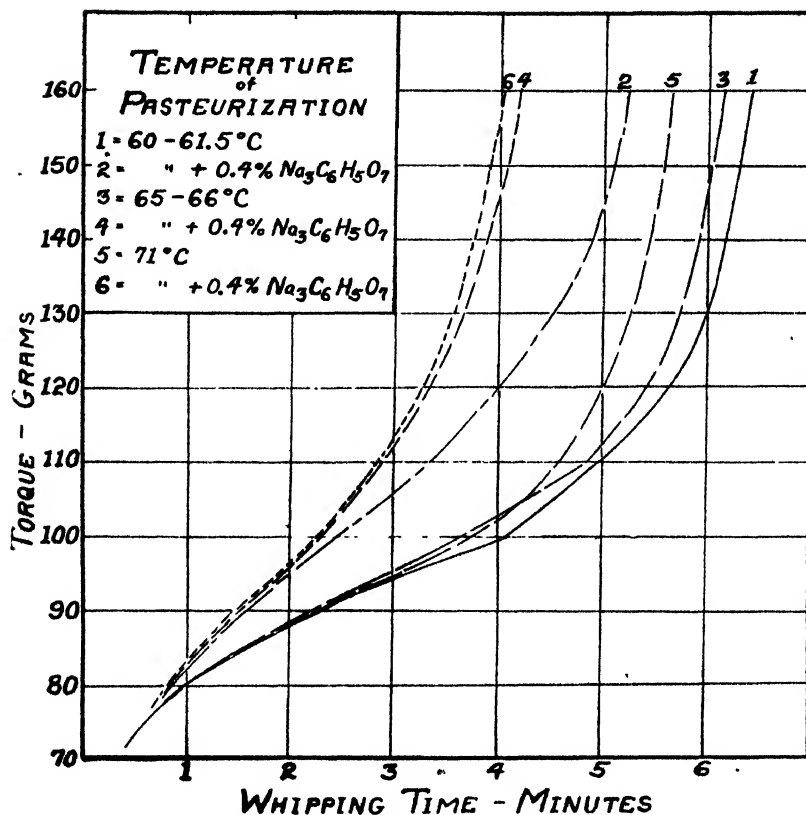


FIG. 3

that the serum remains in the whipped cream when the cream was pasteurized at 70-71° C. This difference can hardly be considered as sufficient to warrant the use of the higher temperature as there is the possibility of the cream getting a slight scorched flavor. It should be noted that all the samples were kept at the temperatures indicated for the full thirty minutes. In the first section of this table the fat content of the cream was too high for the addition of sodium citrate to have any beneficial effect as regards decreasing the whipping time.

After the cream has been pasteurized it is very essential that it be cooled to 15° C. or below before it is put into storage. An example of the effect of poor cooling is given in table 4. In this instance the cream was cooled to 22° C. before it was bottled and placed in the storage room at 6.6° C. While the fat content (31 per cent) of this cream was high enough to warrant a satisfactory whipping quality, and as shown in the preceding table the temperature of pasteurization has little effect upon the whippability, this cream did not whip. As shown in the second part of the table the whipping quality improved after storage for 70 hours. This combination

TABLE 4

Temperature of pasteurization. Effect of poor cooling after pasteurization

PASTEURIZATION TEMP. °C.	WHIP. TIME SEC.	AVERAGE TORQUE INCREASE PER SEC.	OVER- RUN %	DRAIN			
				Time sec.	Weight gms.	Fat %	Fat loss
I. Less than 48 hours' storage at 44° F. Cream containing 31% butterfat							
58-61	360	0.115	134	22	85	17.00	14.79
58-61*	390	0.130	133	37	71	11.25	8.03
64-66	360	0.112	135	26	95	21.00	19.93
64-66*	360	0.140	133	35	64	15.25	10.37
69-71	360	0.119	134	24	83	21.50	18.87
69-71*	318	0.293	144	220	43	9.25	4.04
II. After 70 hours' storage. Cream containing 31% butterfat							
58-61	330	0.390	164	1058	24	3.00	0.71
58-61*	210	0.619	168	839	33	3.25	1.07
64-66	263	0.544	170	668	27	7.00	2.05
64-66*	233	0.531	155	825	29	4.00	1.16
69-71	320	0.401	153	599	17	4.25	0.70
69-71*	203	0.643	154	688	32	5.00	1.63
III. Cream cooled to 2° C. and whipped after eight hours at that temperature. Cream containing 32% butterfat							
No. 1	132	0.808	168	326	45	8.50	3.66
No. 2†	142	0.880	171	293	40	7.70	3.08
No. 3‡	142	0.822	172	483	39	7.20	2.81

* 0.40 % sodium citrate added to these samples.

† 0.055% lactic acid added before pasteurization.

‡ 0.046% citric acid added before pasteurization.

of circumstances certainly indicates that it is quite necessary to cool cream intended for sale as whipping cream to temperature sufficiently low to avoid difficulties. In the regular routine preparation of samples, the cream was cooled to at least 15° C. before it was bottled. In the third section of this table a few data are presented to show the effect of thorough cooling at low temperatures on the whippability of cream. The slight difference in the fat content cannot be given as the reason for the better whipping after such a short period of storage.

It is well known that the whipping quality of ice cream mixes is greatly improved by homogenization and there have been claims made by the manufacturers of homogenizers that whipping cream would whip much more satisfactorily if homogenized at low pressures. The results of the study

TABLE 5
Homogenization

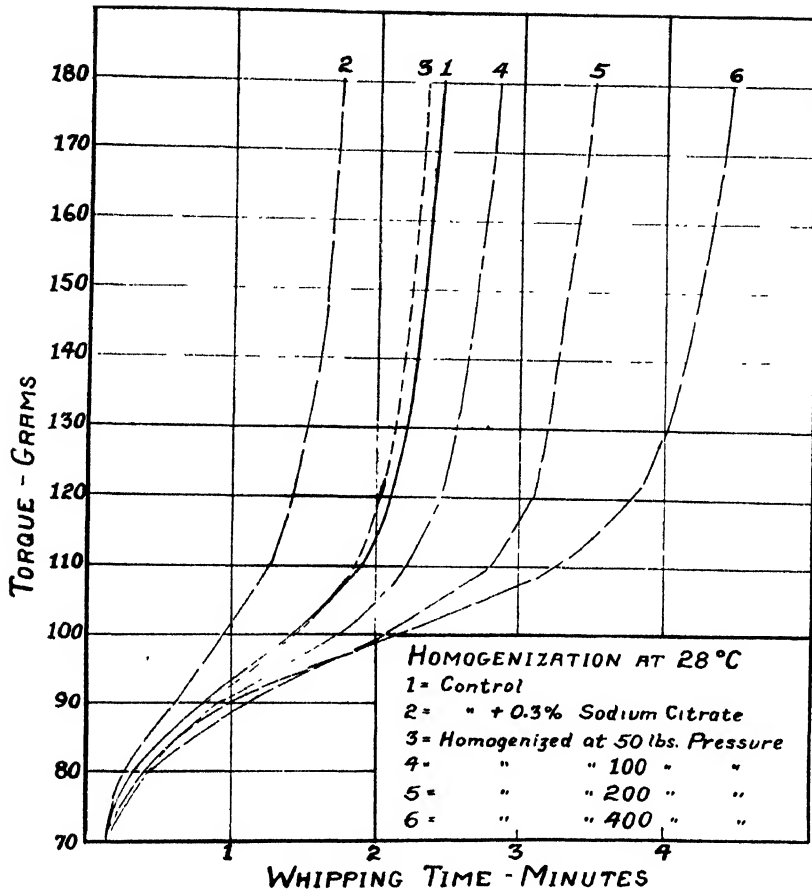
PRESSURE POUNDS	WHIPPING TIME SEC.	AVERAGE TORQUE INC./SEC.	OVER- RUN %	DRAIN			
				Time sec.	Weight gms.	Fat %	Fat loss
I. Above 50° C. Cream containing 30½% butterfat.							
Con.	263	0.469	174	509	51	4.50	2.31
Con.*	183	0.691	167	474	54	3.58	1.95
100	408	0.178	167	166	88	15.75	14.32
100*	340	0.368	175	391	65	9.95	6.26
200	390	0.120	160	54	131	22.25	29.22
200*	402	0.223	171	191	90	16.00	14.74
300	390	0.121	162	47	131	24.25	32.02
300*	383	0.182	180	190	111	19.88	22.54
400	375	0.139	170	61	126	23.50	29.72
400*	390	0.157	176	81	122	22.38	27.48
II. 30 to 37° C. Cream containing 30½% butterfat.							
Con.	199	0.627	172	476	40	5.10	2.03
Con.†	172	0.738	168	399	46	5.00	2.32
100	228	0.548	170	486	44	5.45	2.40
100†	158	0.787	171	555	49	5.15	2.54
200	338	0.366	170	637	49	6.85	3.32
200†	233	0.534	171	627	53	5.90	3.13
300	390	0.140	148	101	100	23.75	23.72
300†	331	0.357	173	689	64	10.75	6.90
400	420	0.123	140	75	106	24.38	25.83
400†	413	0.256	169	324	81	16.38	13.56
III. Below 28° C. Cream containing 32% butterfat							
Con.	146	0.902	162	659	42	4.90	2.04
Con.‡	104	1.262	158	959	42	4.45	1.85
50	140	0.959	160	477	44	4.90	2.15
50‡	132	0.987	157	482	48	6.65	3.22
100	169	0.772	161	633	47	5.88	2.78
100‡	155	0.841	153	511	51	7.08	3.59
200	209	0.622	161	605	47	8.83	4.15
200‡	179	0.730	156	649	53	9.23	4.85
400	266	0.487	160	587	52	11.50	5.94
400‡	202	0.639	157	597	55	10.63	5.87

* 0.40% of sodium citrate added to these samples immediately after homogenization.

† 0.30% sodium citrate added to these samples after homogenizing.

‡ 0.30% sodium citrate added to these samples of cream before pasteurization and homogenizing.

of the homogenization of whipping cream are given in table 5 and figure 4. The cream was homogenized with a two stage machine but using only one stage. A special pressure gauge was used, graduated from 0 to 400 pounds. In the first section of table 5 homogenization was started as soon as the cream was pasteurized, but due to trouble in the adjustment the temperature dropped about 11° C. before all the samples had been obtained. It was found that an attempt to portray the results in the same manner as before gave a number of lines that were almost identical. In view of the



results obtained in the other series of experiments on the effect of homogenization the failure of the sodium citrate to decrease the whipping time at the higher pressures was unexpected and no explanation can be offered at this time.

As compared with the control samples, homogenization had a slight beneficial effect upon the overrun but the slight increase was more than counterbalanced by the longer whipping time and the excessive drainage which had a high fat content. In this connection it is interesting to note the effect of the addition of sodium citrate in increasing the drainage time, decreasing the amount of drainage and also the fat content.

The second section of table 5 is not presented in chart form. In the table it should be noted that the addition of sodium citrate to the samples usually decreased the whipping time, this decrease ranged from 7.3 per cent at 300 pounds pressure to about 31 per cent at 100 and 200 pounds. The longer whipping time for the samples homogenized at 300 and 400 pounds

would lead one to expect the relatively poor overrun with the accompanying high fat loss in the drainage.

The third section of the table is presented in graph form in figure 4. As noted on the table the sodium citrate was added to the cream before pasteurization, and the cream was cooled to 28° C. before homogenization was started. In this series the decrease in the whipping time with the addition of sodium citrate was just the reverse of the preceding being only 5.7 per cent at fifty pounds and 24.0 per cent at 400 pounds. The use of 50 pounds pressure resulted in a fairly satisfactory whipping cream, but unless a producer has the necessary gauges for the homogenizer the problem of regulation is one that will require constant supervision. The overrun of all samples in this series is very uniform, the drain time is also quite uniform among the homogenized samples. In accordance with the results presented in the preceding sections of the table the fat content of the drain and the fat losses increase with the increase in the pressure applied to the cream during homogenization. As a generalization it might be said that the addition of 0.30 per cent of sodium citrate is equivalent to a reduction of 100 pounds pressure in the whipping time of the cream.

SUMMARY

The reaction of normal sweet cream is probably the most satisfactory for the production of a good whipping cream.

If the cream has developed a slight acidity, but not enough to affect the taste it is better to pasteurize it at once rather than neutralize and then pasteurize.

The addition of small amounts of acid (less than 0.03 per cent) does not have an appreciable effect upon the cream. If the titrable acidity calculated as lactic acid is above 0.27 per cent the cream will whip rapidly, but such a cream is eliminated from practical consideration because of its sour taste and the low overrun and soggy appearance of the whipped product. Fat losses in the cream decrease with increasing acidity.

Cream separated at temperatures of 60 to 72° F. (15.5 to 22.2° C.) and 150° F. (65.6° C.) whipped better than creams separated at intermediate temperatures. The lower separating temperatures gave better results as regards the amount of serum lost and its fat content.

The temperature of pasteurization is not an important factor as far as whipping quality is concerned. The usual temperatures are very satisfactory.

Cream intended for whipping must be thoroughly cooled before it is bottled and placed in storage for aging.

Poorly cooled cream showed a marked increase in whippability after storage for more than 70 hours at 44° F. (5.6° C.).

Cream should not be used for whipping until it has aged for 24 hours. If it is necessary to use the cream after a shorter period of aging, the temperature of storage should be as close to 0° C. as possible.

Homogenization of the cream does not have a beneficial effect. If the cream is homogenized it should be done at a low temperature and the pressure should not exceed 100 pounds. It is essential that the gauges be watched continually to avoid changes in pressure. The addition of sodium citrate to the cream before pasteurization decreases the whipping time; the same effect was noted when sodium citrate was added immediately after homogenization. Fat losses in the drainage increase markedly with increasing homogenization pressures.

In conclusion the authors wish to express their thanks to Chas. Pfizer and Co., Inc., who have sponsored the fellowship that has made this study possible.

FACTORS INFLUENCING THE INITIAL INDUCTION PERIOD IN THE OXIDATION OF MILK FAT¹

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Flavor defects in milk and cream which have been described as "oxidized," "cardboard," "cappy," or "metallic" have been observed for some time in milk and cream which have been exposed to sunlight or contaminated by certain corrosive metals such as copper and some of its alloys. It has been reported by a number of investigators that the change in flavor is caused by oxidation of the milk fat. The active oxidation of a fat is preceded by a period of varying length in which oxygen is not absorbed. This period depends on the chemical constitution of the fat, temperature of holding the fat during processing, storage and exposure to catalysts. This period is known as the induction period and is a measure of the stability of the fat. This paper is concerned with the measurement of the influence of sunlight, certain metals and submaintenance rations of the cow, on the initial induction period in the oxidation of milk fat.

Hammer and Cordes (1) were, apparently, the first to report that exposure of milk to sunlight produced tallowy flavors. The effect was determined organoleptically. This observation was confirmed by Frazier (2) and Tracy and Ruehe (3). Frazier further postulated that the presence of a metal catalyst, resulting from contact with equipment or other sources, would, undoubtedly, assist the catalytic action of daylight. Tracy and Ruehe concluded from their work that "diffuse light is an important factor in the development of tallowy flavors in milk, especially that containing an added copper salt." The influence of sunlight and metals on the induction period were not determined by these investigators. Anderson and Triebold (4) in studying the influence of irradiation of butter on the fat constants and keeping quality of milk fat found that irradiation of a sample of butter reduced the induction period approximately 25 per cent.

Many experiments have been reported where copper and certain other metals corroded by milk are said to have caused "oxidized" flavor in milk (5). The method used in the experiments referred to, however, was organoleptic. No work so far as our search of the literature has been able to disclose, has been reported on the influence of pasteurizing cream in the pres-

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ence of copper and other metals upon the induction period of the milk fat. Briggs (6) and Wright (7) added different metallic salts to butterfat and other fats and determined the influence of such treatment on the induction period. The adding of metallic salts directly to fat, however, is not comparable to pasteurizing the cream in contact with the metal. The greater reduction of the induction period when metallic salts were used, as shown by both Briggs and Wright, bears out this last statement.

EXPERIMENTAL

Briggs (6) and Wright (7) determined the induction period by the oxygen absorption method. Greenbank and Holm (8) used this method in their earliest work and later developed the photochemical method (9). This latter method consists of the observation of the decolorization of methylene blue in pure dry milk fat by means of a photoelectric cell and appropriate accessory apparatus. Greenbank and Holm conclude from their work in developing the method that the rate of reduction of methylene blue in a fat or oil when catalyzed by light served as a measure of the rate of reaction of the initial oxidative processes and that it could be utilized to determine the relative susceptibilities of fats and oils to oxidation. Royce (10) used the method with certain modifications in a study of the "rancidity" of cottonseed oil. The apparatus used in the work reported in this paper (Fig. 1) was essentially like that of Greenbank and Holm (9) with the following modifications.

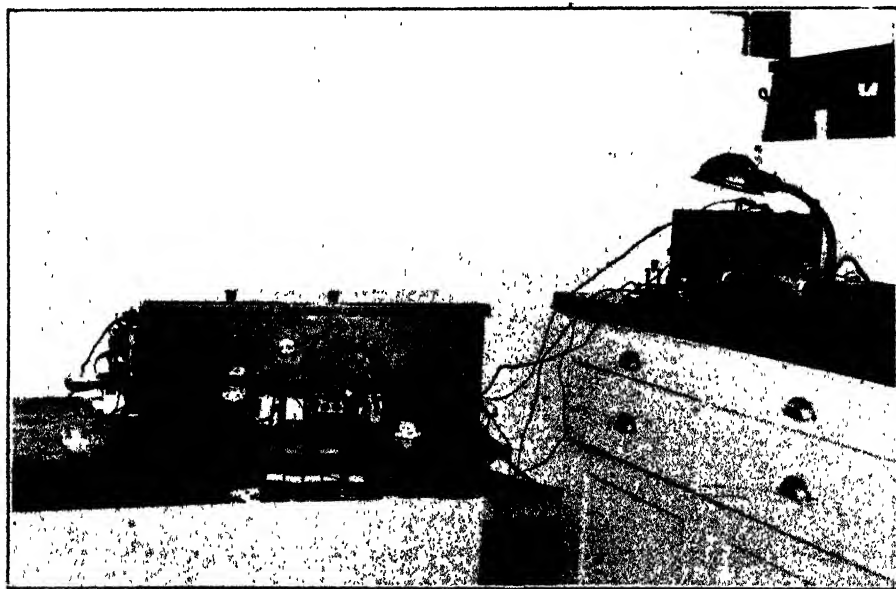


FIG. 1

1. The reaction cell was redesigned in order to permit the constant stirring of the fat during the test (Fig. 2). This latter was found necessary in order to insure uniform fading of the methylene blue.

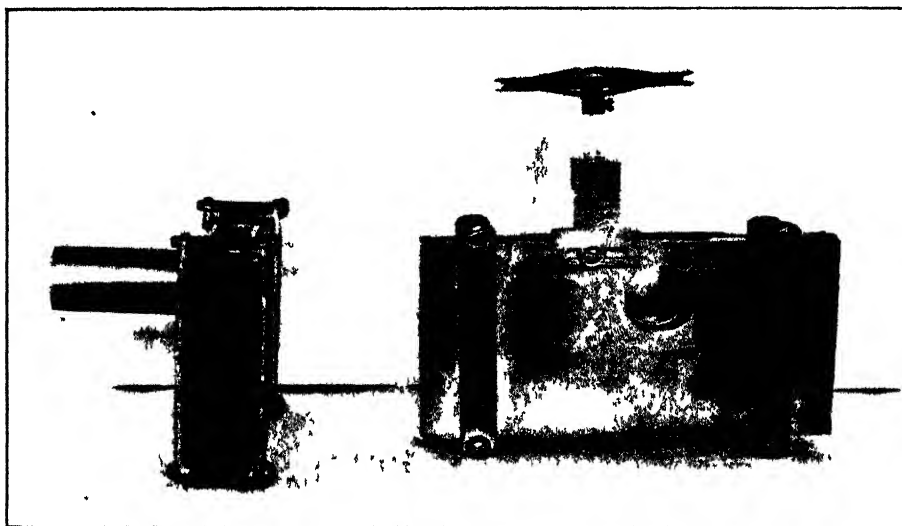


Fig. 2

2. It was found that a 100 watt-110 volt A. C. Mazda lamp could not be used under the operating conditions available. The variations in line voltage caused excessive variations in the intensity of the light, and, hence, in the output of the photo-cell. A constant light intensity is necessary in using a photoelectric cell to detect color changes. A Leeds & Northrup 12-16 volt current regulator and a 50 candle power 12 volt automobile lamp were found to give a constant light source.

3. It was found that the addition of the methylene blue solution permanently changed the color of the fat and that after several hours in the test apparatus the amount of light passing through the fat never equalled that of the pure fat to which methylene blue had not been added. Visual fading of the fat occurred when the amplified output of the cell had increased approximately 1.5 milliamperes. For the conditions described in this experiment an increase of 1.5 milliamperes was arbitrarily chosen as the end-point of the fading of methylene blue. This end-point made the readings comparable when samples of the same fat, treated differently, were compared with each other and when the amount of methylene blue and the distance of the light from the reaction cell were kept constant.

4. A forward-connected photo-electric cell current circuit (Fig. 3) was used in place of the reverse connected circuit used by Greenbank and Holm and by Royce. This circuit is so designed that an increase in light falling upon the cell produces an increased output shown on the milliammeter.

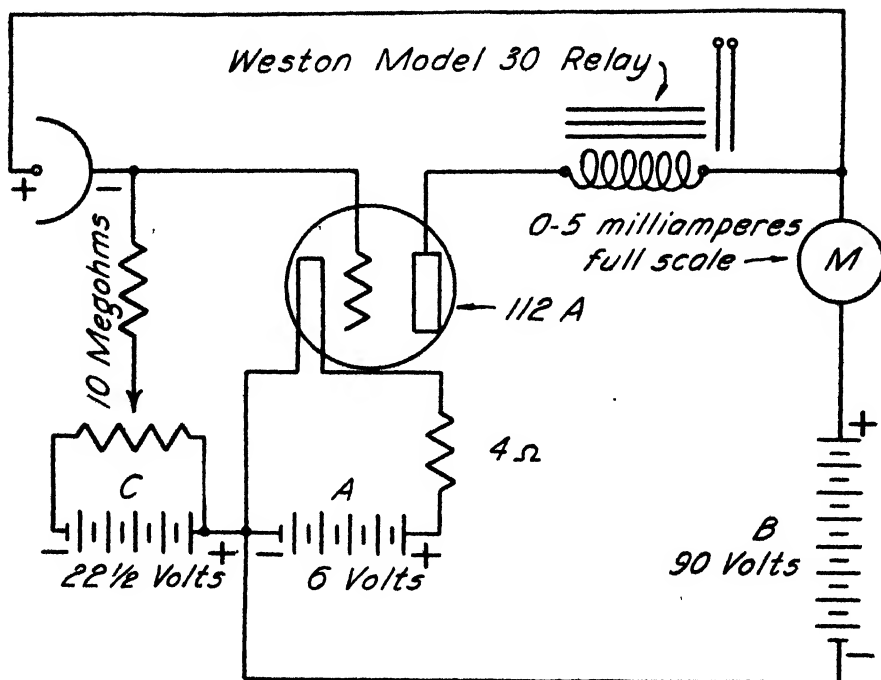


FIG. 3

Preparation of experimental material: In preparing the fat samples for testing, the cream and milk from which it was separated were protected from light, high temperatures and metals corroded by milk, in order that the induction period of the control samples would represent the true susceptibility of the fat to become oxidized. The cream was churned in a dark room in a glass churn. The butter was melted and filtered in a 45° C. oven and then stored at -5° C. and protected from light until the tests were made in the apparatus.

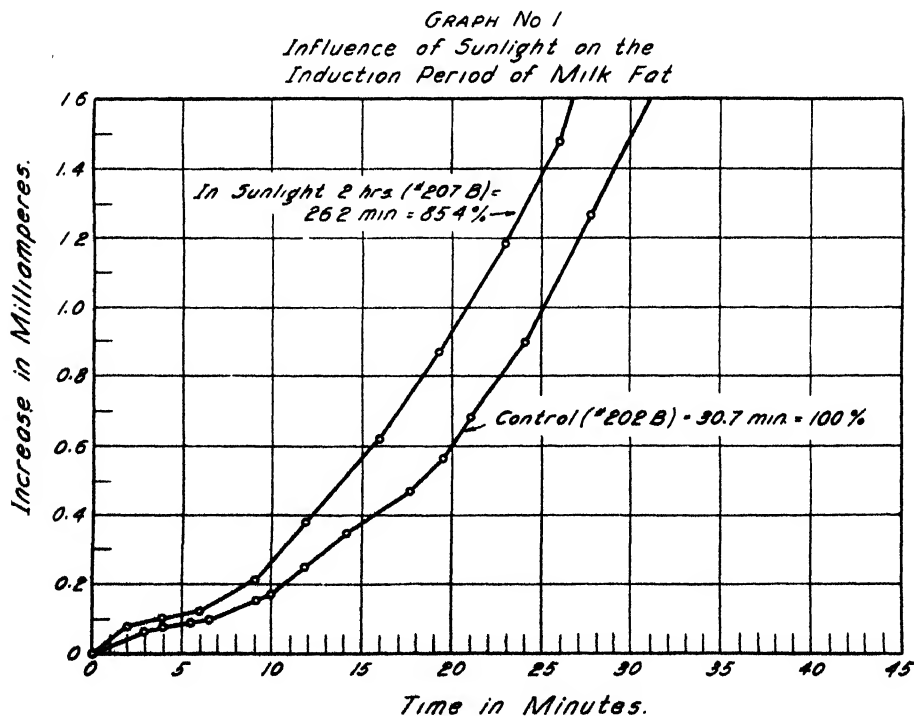
When it was desired to test the effect of sunlight or metals on the induction period, the milk was handled as previously described up to the point of churning. If metals were to be tested, 1" x 2" strips of the metals were placed into 750 cc. of cream and the cream pasteurized. Heating to 145° F., holding for 30 minutes and cooling at 40° F. required approximately one and one half hours. Control samples were pasteurized in the same water bath with the samples containing the metal strips. Cream samples which were exposed to sunlight were unheated. Raw samples of the same cream were protected from light and used as controls.

Conducting the tests: The tests to determine the induction periods were made in the following manner: Twenty-five cc. of the pure dry milk fat were melted and heated to approximately 65° C. One and one half cc. of a 0.025 per cent solution of methylene blue in absolute alcohol were then added to the

fat. A pipette was used to transfer approximately twenty cc. of the fat and dye mixture to the reaction cell (Fig. 2). The light in the right compartment was then turned on and readings on the milliammeter taken at intervals until the reading had increased 1.5 milliamperes over the original reading. This reading was taken as the end-point of the fading of the methylene blue and the time required to reach the end-point is reported as the induction period. Graphs 1 to 4 give some of the data in the form of curves. The position of the light and the photo-cell were kept constant. The results indicate that the maximum variation of duplicates in fading time of the methylene blue is approximately 3 per cent.

RESULTS

Graph 1 gives the results of exposing raw sweet cream which had been exposed to direct sunlight for two hours together with the control sample of the same cream which had been protected from light. The curve of Sample 202B shows the rate of reduction of the methylene blue in the control sample which has been arbitrarily designated as 100 per cent. The curve of Sample 207B gives the induction period of the treated sample which was 85.4 per cent of that of the control sample, or, in other words, direct sunlight, under the conditions of the experiments, markedly increased the sus-

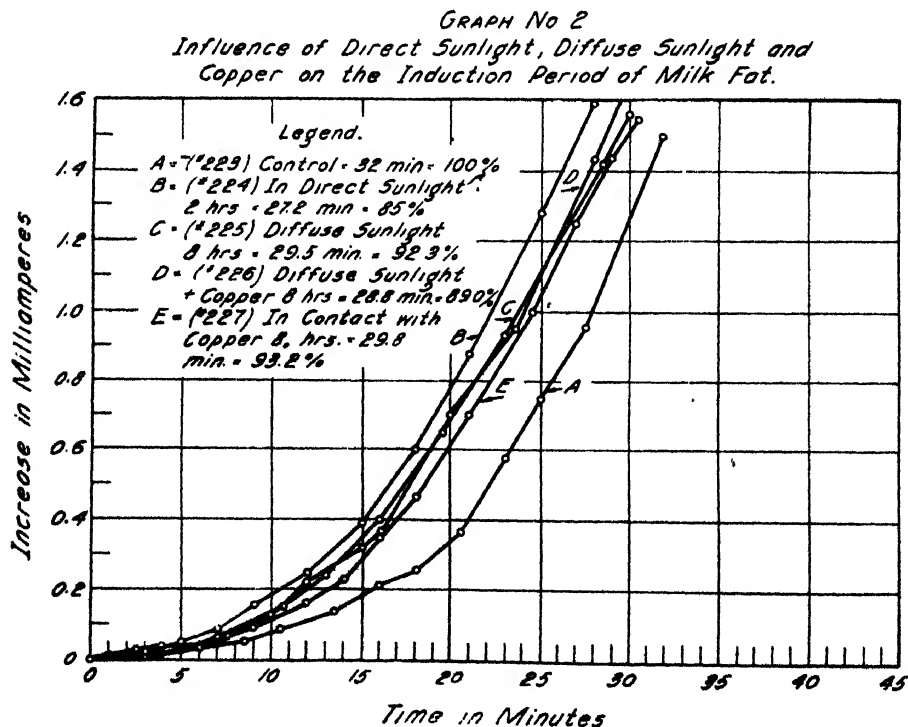


ceptibility of milk fat to oxidation. The flavor of the cream which had been exposed to direct sunlight was distinctly that of oxidized fat.

Graph 2 gives the induction period of raw fresh cream under the following conditions:

Curve A is the control sample which has been arbitrarily designated as	100%
Curve B direct sunlight 2 hours	85%
Curve C diffuse sunlight 8 hours	92.3%
Curve D diffuse sunlight 8 hours with copper	89.0%
Curve E treated as in Curve D except protected from light	93.2%

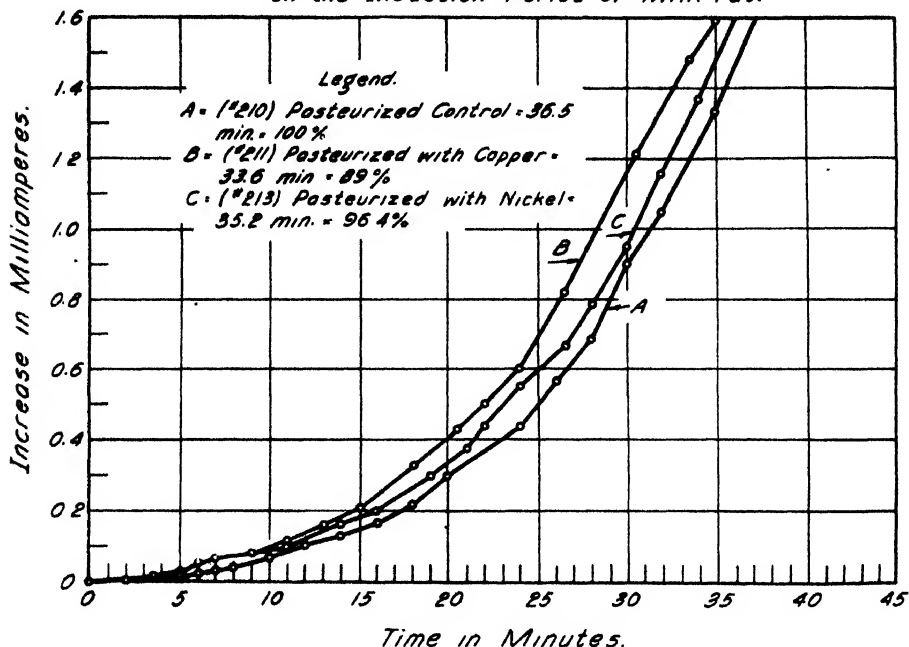
This graph shows that exposure of cream to direct sunlight decreased the induction period to a greater extent than did its exposure to diffuse sunlight, to diffuse sunlight and copper, and to copper alone.



Graph 3 gives the results of pasteurizing two samples of cream, one in contact with copper and the other with nickel. Curve A shows the induction period of the control sample which is arbitrarily considered at 100 per cent. Curve B gives the induction period of the sample pasteurized with copper which was 89 per cent of the control. Curve C gives the induction period of the sample pasteurized with nickel which was 96.4 per cent of the

control. From Curve B one would conclude that copper had a marked influence in reducing the induction period of fat at while nickel, as shown in Curve C, had only a slight influence.

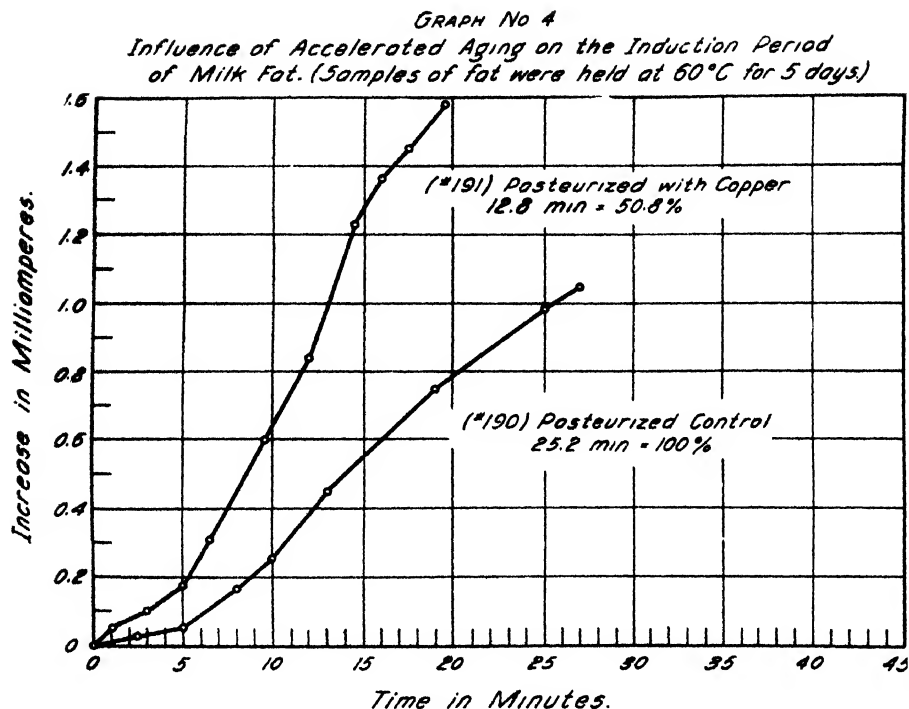
GRAPH No. 3
Influence of Copper and Nickel
on the Induction Period of Milk Fat.



Graph 4 gives the results of an accelerated aging test of a control and a sample pasteurized with copper. To accomplish this, the pure fat samples were held in a 60° C. oven for five days. The curve of Sample 191 shows the induction period of the sample pasteurized with copper. The induction period was 50.8 per cent of that of the control sample. These results show that cream pasteurized in contact with copper produces pure fat that is markedly more susceptible to oxidation than fat that is not exposed to copper. In another accelerated aging test, using a different fat, the induction period was reduced to 75.3 per cent of the control.

Cream samples pasteurized in contact with a chrome-nickel-iron alloy of the 18-8 series with surface finishes, designated No. 1, No. 2, and No. 4, were tested and found to have no influence in changing the induction period of milk fat.

The influence of unsaturation of milk fat on the induction period: Hunziker, Mills and Spitzer (11) have shown that feeding cows rations high in linseed oil meal or cottonseed oil meal will greatly increase the iodine number of fat. Frazier (2) has fed cows such rations and has found that the milk



did not become tallowy more rapidly or to a greater extent when exposed to sunlight than milk produced from cows on rations that did not contain these ingredients. Triebold and Bailey (12) reporting their work on shortenings conclude "on the basis of the induction period, the susceptibility of shortenings to oxidative rancidity appears to be related to the unsaturation of the fatty acid glycerides." Eckles and Palmer (13) were able to increase the iodine number of milk fat approximately 15 units by feeding the cows on subnormal rations. No information was found in the literature that reported the change in induction period accompanying such a change in unsaturation of the milk fat.

Two cows (No. 403 and No. 478) of the Station herd were maintained on normal rations with the required amount of nutrients for milk production. The cows were in the third month of lactation, and were yielding approximately 40 pounds of milk per day. After samples were collected and tested for the normal period, the cows were reduced to submaintenance rations of 12 pounds of alfalfa hay per day. After 48 and 60 hours of this régime, other samples were collected for analysis. The cows dropped approximately 40 per cent in milk yield and showed loss in weight. The iodine numbers of the milk fat produced during these two régimes are given in table 1.

TABLE 1

Iodine numbers of milk fat produced from cows on normal and submaintenance rations

COW NUMBER	SAMPLE NUMBER	IODINE NUMBER	RÉGIME
403	208	31.1	Normal
403	200	48.1	Subnormal
478	209	36.6	Normal
478	201	54.2	Subnormal

The iodine numbers of the fats from Cows No. 403 and No. 478 were increased 17 and 17.6 units respectively during the submainaenance régime. The cows were apparently drawing upon their body fat while the submain-tenance rations were being fed.

The induction periods of the fat secured from the milk produced from Cow No. 478 during the periods of normal and subnormal feeding were determined. These data are shown below:

TABLE 2

SAMPLE NUMBER	RÉGIME	IODINE NUM- BER (HANUS)	INDUCTION PERIOD	PERCENTAGE OF NORMAL
209	Normal	36.6	27 min.	100.
201	Submaintenance	54.2	20 min.	74.2

It is evident from these results that the increase in the iodine number (Hanus) is accompanied by a shorter induction period. The iodine number was increased from 36.6 to 54.2 and the induction period was reduced 25.8 per cent. It would appear that any condition or feed that will greatly increase the unsaturation of milk fat will increase the susceptibility of the fat to become oxidized. The condition reported is an extreme one and under ordinary herd management would not be encountered to the same extent.

CONCLUSIONS

(1) The susceptibility of milk fat to oxidation due to different conditions may be measured by the photochemical method described by Greenbank & Holm with such modifications as have been described in this paper.

(2) Exposure of cream to direct sunlight, to diffuse light and to the action of copper gave definite increases in the susceptibility of milk fat to oxidation and the extent of the change was indicated by the method used. Direct sunlight, apparently, had the greatest influence. Nickel showed a slight influence, and a chrome-nickel-iron alloy of the 18-8 series had no influence on the susceptibility of milk fat to become oxidized.

(3) Milk produced from animals drawing upon their body fat by the consumption of submaintenance rations showed increases in the percentages of unsaturated fats, and increased susceptibility of the fat to oxidation.

REFERENCES

- (1) HAMMER, B. W., AND CORDES, W. A. A study of brown glass milk bottles. Res. Bul. 64, Iowa Agr. Expt. Sta. 1920.
- (2) FRAZIER, W. C. Defect in milk due to light. JOUR. DAIRY SCIENCE 11: 375-379. 1928.
- (3) TRACY, P. H., AND RUEHE, H. A. Flavor development in market milk. JOUR. DAIRY SCIENCE 14: 250-267. 1931.
- (4) ANDERSON, A. K., AND TRIEBOLD, H. O. A biochemical study of irradiated milk. JOUR. DAIRY SCIENCE 15: 469-474. 1932.
- (5) GUTHRIE, E. S., ROADHOUSE, C. L., AND RICHARDSON, G. A. Corrosion of metals by milk and its relation to the oxidized flavors of milk. Hilgardia 5: 425-463. 1931. (Contains bibliography of 35 references on the subject.)
- (6) BRIGGS, L. II. The autoxidation of butterfat. I—Factors influencing the reaction. II—Comparison of tests for detecting oxidation changes. Jour. Dairy Res. 3: 61-79. 1931.
- (7) WRIGHT, K. E. A study of some of the factors affecting the autoxidation of butterfat. Master's Thesis, University of Illinois. 1927.
- (8) GREENBACK, G. R., AND HOLM, G. E. Measurement of the susceptibility of fats to oxidation. Jour. Ind. & Eng. Chem. 17: 625. 1925.
- (9) GREENBACK, G. R., AND HOLM, G. E. A photo-chemical method for measuring susceptibility of fats and oils to oxidation. Jour. Ind. & Eng. Chem. (Analytical Edition) 2: 9-10. 1932.
- (10) ROYCE, H. D. An index of rancidity by methylene blue reaction. Soap 7: 25-27. 1931.
- (11) HUNZIKER, O. F., MILLS, H. C., AND SPITZER, G. Moisture control of butter. Indiana Expt. Sta. Bul. 159. 1912.
- (12) TRIEBOLD, H. O., AND BAILEY, C. H. I—Autoxidation of shortenings and crackers. II—Factors influencing the keeping quality of shortenings and crackers. Cereal Chem. 9: 50 & 91. 1932.
- (13) ECKLES, C. H., AND PALMER, L. S. The influence of the plane of nutrition of the cow upon the composition and properties of milk and butterfat. University of Missouri Agr. Expt. Sta. Res. Bul. 25. 1916.

THE VALUE OF HAND STRIPPING AFTER MACHINE MILKING¹

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INTRODUCTION

Hand stripping has been one of the most serious objections raised by dairymen to the use of milking machines. Petersen and Swenson (3) and Wallis *et al.* (5) have pointed out that little information is available as to whether or not stripping is necessary to maintain production. With the use of the combine milker, hand stripping is very inconvenient since the milk is handled entirely by machine. Some users have dispensed with hand stripping with apparently little ill effects. Others have massaged the udder while the machine was still operating in order to remove the last of the milk. The work reported here was carried out during the winter of 1931-32 to investigate this problem from three angles:

1. To find the amount of time spent in stripping and the amount of milk and fat secured in the strippings following machine milking.
2. To determine the effect of omission of hand stripping upon the production of machine milked cows.
3. To determine to what extent the amount of strippings can be decreased by procedures designed to increase the thoroughness of machine milking.

EXPERIMENTAL

I. Time Required to Strip after Machine Milking and the Amount of Milk and Fat in the Strippings²

The time required to strip was determined for each of 25 cows. The amount of milk and fat was found in the strippings of 23 of them. The group included 12 Holsteins, 5 Jerseys, 3 Guernseys, 4 Brown Swiss and 1 Ayrshire. The cows varied in age and stage of lactation, and their production ranged from 11 to 63 pounds of milk per day. The cows were milked twice daily. The milking machine was left six minutes on each cow. Previous work at this Station (2) has shown this to be about right for heavy milking cows. The time required to strip each cow was recorded for five or more milkings and averaged. Strippings were weighed and tested during a period of five days.

An average of 47 seconds per milking was required to strip after the machine. This time varied from 25 to 81 seconds with individual cows.

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¹ Journal paper No. J122 of the Iowa Agricultural Experiment Station, Ames, Iowa.

² The machine used in this trial was a De Laval Magnetic Milker.

The average amount of strippings obtained was 0.6 of a pound per milking or 1.2 pounds per day. Some cows gave as little as 0.1 of a pound per milking while the heaviest stripper averaged 1.7 pounds per milking. Between cows the amount of strippings was not closely related to the amount of milk produced, but with the same individual the amount of strippings tended to increase with advancing lactation. The fat test of the strippings of individual cows ranged from 2.8 to 13.5 per cent, with an average of 7.5 per cent for the 23 cows. Four and two-tenths per cent of the total milk produced and 7.3 per cent of the total fat produced were obtained in the strippings.

TABLE 1

Milk and fat production of cows not stripped after machine milking as compared to their production when stripped after machine milking

PRODUCTION		PERIOD I	PERIOD II	PERIOD III
Group A (5 cows)		Stripped	Not stripped	Stripped
	Milk lbs.	7773.0	6649.0	6068.0
	Fat lbs.	279.5	237.2	220.4
	Per cent fat	3.60	3.57	3.63
Group B (3 cows)		Not stripped	Stripped	Not stripped
	Milk lbs.	3800.0	3564.0	3359.0
	Fat lbs.	146.7	134.3	128.4
	Per cent fat	3.86	3.77	3.82

Summary of data

PRODUCTION OF 8 COWS IN 40 DAYS	MILK, LBS.	FAT, LBS.	PER CENT FAT
<i>Stripped</i>			
Av. of Group A in Per. I & III plus Group B in Per. II	10,485	384.2	3.66
<i>Not stripped</i>			
Av. of Group B in Per. I & III plus Group A in Per. II	10,229	374.8	3.66
Increase in production during period of stripping	256	9.5	
Av. increase per cow per day during period of stripping	.80	.03	
Per cent increase during period of stripping	2.5	2.5	

II. The Effect of Not Stripping after Machine Milking

Ten cows were divided into two groups as uniform as possible in regard to amount of strippings, total milk and fat production, breed, age, and stage of lactation. During the trial two cows were dropped from one of the groups for reasons not connected with the experiment, leaving only three animals in that group. The trial consisted of three 40-day experimental periods separated by transition periods of 5 days each. During each 40-day period the production of one group of cows which was not stripped after the milking machine was compared with that of the other group which was

hand stripped after the machine. The groups were alternated by the double reversal method. Each animal was stripped as soon as the milking machine was removed. One man did all the stripping to ensure uniformity. The machine-drawn milk and strippings of each cow were weighed, sampled and tested for butterfat separately at each milking during the first ten days of each 40-day period. During the latter 30 days of each period strippings were added to the machine-drawn milk and the tests were based on 5-day composite samples. All cows were stripped during the transition periods and weights and tests were taken at each milking. The milk and strippings were weighed to the nearest tenth of a pound on spring balance scales. A sampling tube was used for taking the composite samples. Fat tests were determined by the Babcock method.

The production of each group of cows during periods of stripping and not stripping are shown in table 1. The data were summarized by comparing the average of the first and third 40-day periods of the double reversal trial with the second. During the 40-day periods in which stripping was practiced the total daily production per cow amounted to 0.8 of a pound more milk and 0.03 of a pound more fat than during the corresponding period in which stripping was omitted. This was equal to an increase of 2.5 per cent in milk and fat production. The average butterfat test of the milk showed no change due to failure to strip. Figure 1 presents graphically the production records of the cows during periods of stripping and not stripping, and also during the 5-day transition periods previous to and following the 40-day period.

III. Practices Designed to Increase the Thoroughness of Machine Milking

Two methods of manipulation were tried in an effort to reduce the amount of strippings retained in the udder. The first consisted of thoroughly massaging the udder during the last two minutes of operation of the machine (the machine remained on each cow six minutes). In this method the teat cups or claw of the machine were not disturbed. The second method, designated in this article as manipulation of the teat cups, consisted of grasping the claw of the machine with the hand and exerting as much downward pressure during the last minute of milking as was possible without pulling the cups off the teats. This downward pressure was released at intervals, but no attempt was made to massage the udder further than resulted from the application and release of pressure on the teat cups.

The effect of massaging the udder was determined in a trial with three periods of 10 days each. Two groups of five cows each were used, the udders of one group being massaged while those of the other group were not. The groups were alternated by the double reversal method. Results were calculated by comparison of the average production of each group in

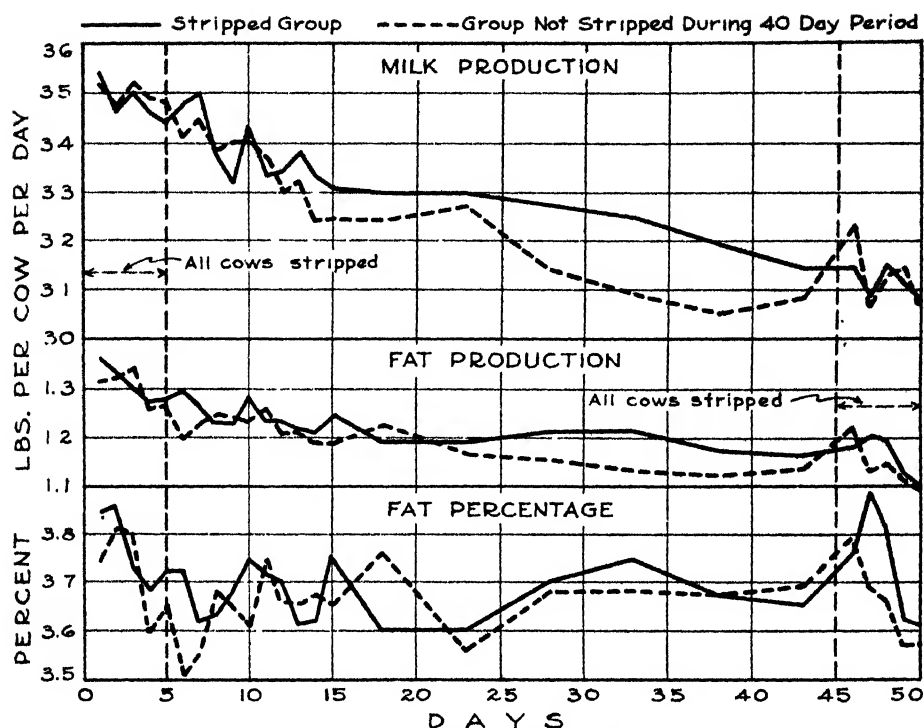


FIG. 1. THE AVERAGE DAILY PRODUCTION OF GROUPS OF 8 COWS STRIPPED AND NOT STRIPPED AFTER MACHINE MILKING.

Note: These figures represent the weighted average production during periods of stripping and not stripping. The production of the cows during the five days preceding and the five days following the 40-day period are also included.

TABLE 2

The thoroughness of machine milking as influenced by massaging the udder while the machine was operating

	GROUP (5 COWS IN EACH GROUP)	PRODUCTION OF GROUPS DURING EACH 10-DAY PERIOD								
		PERIOD I			PERIOD II			PERIOD III		
		Milk lbs.	Fat lbs.	% fat	Milk lbs.	Fat lbs.	% fat	Milk lbs.	Fat lbs.	% fat
Machine-drawn milk	A	1117	44.6	3.99	1028	41.8	4.07	1025	41.6	4.06
	B	1073	42.1	3.93	1051	41.6	3.94	1007	40.9	4.06
Strippings after machine milking	A	41	3.3	8.15	46	3.5	7.54	35	3.0	8.37
	B	55	3.3	6.03	26	2.2	8.34	41	2.9	7.02
Total production	A	1158	47.9	4.14	1074	45.3	4.22	1060	44.6	4.21
	B	1128	45.5	4.03	1077	43.8	4.07	1048	43.8	4.18

Group A massaged in periods I and III but not in period II.

Group B not massaged in periods I and III but massaged in period II.

Summary of data

		AV. PRODUCTION PER COW PER DAY LBS.		PER CENT OF TOTAL PRODUCTION	
		Massaged*	Not** massaged	Massaged	Not massaged
Machine-drawn milk	Milk	21.2	20.7	97.1	95.7
	Fat	.847	.833	94.1	92.7
	% fat	3.99	4.03		
Strippings after machine milk- ing	Milk	.64	.94	2.9	4.3
	Fat	.054	.066	5.9	7.3
	% fat	8.29	6.98		
Total production	Milk	21.9	21.6		
	Fat	.901	.899		
	% fat	4.12	4.16		

* The group which was massaged includes the average of group A in periods I and III plus group B in period II.

** The group which was not massaged includes group A in period II plus the average of group B in periods I and III.

the first and third periods with that of the same group in the second period. Table 2 shows the average daily production of milk and strippings for the 10 cows during periods in which massaging was practiced and also during comparable periods when massaging was omitted.

A different method was used to determine the influence of manipulation of the teat cups on the thoroughness of machine milking. Ten cows were used in the trial which covered 15 days. The pail of the milking machine was suspended from a spring balance scale. During a 5-day preliminary period the amount of milk secured by the machine for each successive minute of milking was recorded. At the end of six minutes the milking machine was removed and the cow stripped. During the second 5-day period the machine was operated normally for the first five minutes but during the sixth minute the teat cups were manipulated. The weights of the milk secured by the machine during each successive minute and the weight of the strippings were recorded. In the third 5-day period the machine was operated normally for four minutes, then the teat cups were manipulated during the fifth minute. The machine was removed at the end of five minutes instead of at the end of six minutes. Weights of milk and strippings were recorded as in the preceding periods. Table 3 shows the average pounds of milk secured during each minute of milking and the amount of strippings obtained for each of the three periods.

DISCUSSION OF RESULTS

In this trial it was found that stripping after machine milking resulted in increased production. The average increase during the periods in which

TABLE 3

*The effect of manipulation (pulling down) of the teat cups on the completeness of machine milking**

		AMOUNT OF MILK OBTAINED BY MILKING MACHINE DURING SUCCESSIVE MINUTES OF OPERATION						STRIP- PINGS AFTER MACHINE MILKING	TOTAL PRODUC- TION
		Minutes							
		1	2	3	4	5	6		
Normal machine milking	Milk lbs.	3.2	4.7	3.1	1.3	.4	.2	.5	13.3
	% of total production	23.9	35.0	23.0	9.7	3.3	1.3	3.8	
Teat cups manipu- lated during 6th minute	Milk lbs.	3.1	4.5	2.7	1.3	.5	.7	.2	13.1
	% of total production	23.7	34.5	20.5	10.0	3.7	5.7	1.7	
Machine left on only 5 minutes. Teat cups manipu- lated during the 5th minute.	Milk lbs.	3.2	4.5	2.6	1.2	1.1		.3	12.9
	% of total production	25.2	34.6	20.1	9.6	8.4		2.2	

* Average pounds of milk per cow per milking. Each figure is the average production of 10 cows over 10 milkings.

stripping was practiced was 0.8 of a pound of milk and 0.03 of a pound of fat per cow per day. The average time spent in stripping a cow was 1.57 minutes per day. This means that for each hour spent in stripping the milker received 1.16 pounds of butter fat.

It must be pointed out that only relatively short periods (40-day) of non-stripping were used in this trial. What the effect would have been over a whole lactation period cannot be definitely stated. It may be pointed out, however, that the production was decreased more during the latter part of the 40-day period than at the beginning of the period (Figure 1). Also, during the five days after the resumption of stripping the production of the previously non-stripped group practically paralleled that of the other group. These facts indicate that the loss by not stripping over a whole lactation might be greater than for the 40-day period. On the other hand, Woodward (7) found that incomplete milking did not lead to a rapid drying-off of the cows. He also reports that it did not alter the test of the milk which agrees with the results obtained in this trial.

Woll and Humphrey (6), although declaring that stripping is advisable, report that cows when not stripped for 12 weeks decreased in production no more rapidly than did cows which were stripped. Further work upon the effect of non-stripping is desirable before making too definite a statement regarding the ultimate effect of non-stripping upon production.

At least part of the milk and fat left in the udder when stripping is not practiced is recovered at the succeeding milking. This is shown by the

fact that milk and fat production were increased only 2.5 per cent by stripping, whereas the strippings left in the udders of the eight cows used in this trial constituted 4.4 per cent of the total milk production and 8.5 per cent of the total fat production. The recovery amounted to 46 per cent of the milk and 73 per cent of the fat left in the udder. Probably all the residual milk is actually recovered at the next milking but the presence of the milk causes the pressure to build up more rapidly in the udder. This increased pressure would depress secretion during the interim between milkings since the rate of secretion has been found by Ragsdale *et al.* (4) to be governed by the pressure in the udder. This conclusion is supported by the fact that the test of the milk is not altered by failure to strip and that a larger proportion of fat than of milk in the strippings was recovered.

M'Candlish (1) voices a popular opinion when he suggests that if a cow is stripped after machine milking she may retain a portion of her milk for the stripper instead of milking out completely with the machine. Results secured in this investigation do not bear out this statement. In fact, after a 40-day period without stripping the cows gave an average of 0.3 of a pound more strippings per day than after periods in which stripping was practiced. Merely omitting the stripping process for 40 days did not cause the cows to milk out more completely with the machine.

Certain difficulties encountered in this trial indicate that stripping after machine milking should be recommended as a safety factor. Three times during the trial the machine, due to improper attachment, failed to milk one quarter of the udder. This failure occurred even though a careful milker was operating the machine. By stripping after machine milking this failure was detected, but if stripping had been omitted it might have been overlooked. One cow was also encountered which did not milk readily by machine due to the partial obstruction of one teat. This cow gave as much as five pounds of strippings at a milking. Such a cow must, of course, be stripped after the milking machine. No serious cases of mastitis were encountered among the unstripped cows.

Though the trials relating to substitutes for hand stripping were not very extensive, they indicate that certain procedures will partially replace hand stripping. Manipulation of the teat cups for one minute reduced the amount of strippings to less than half the normal amount. Where stripping is very inconvenient manipulation would render machine milking more thorough and at the same time lessen the chance of mechanical failures of the machine escaping detection. However, neither process investigated was successful in securing all the milk that would have been obtained by stripping. Probably a combination of pulling downward on the teat cups and massaging the udder at the same time would prove more effective than either method used singly, but this was not attempted in the trial being reported.

SUMMARY

Stripping after machine milking required an average of 1.57 minutes per cow per day. One and two-tenths pounds of milk and 0.09 of a pound of fat, representing 4.2 per cent and 7.3 per cent respectively of the day's total production were secured in the strippings.

During periods in which stripping after machine milking was practiced the production of milk and fat was 2.5 per cent greater than in periods in which the stripping was omitted. Calculations show that not stripping resulted in the loss of 54 per cent of the milk and 27 per cent of the fat that would have been obtained in the strippings. No change in the fat percentage of the milk was caused by not stripping. For each hour of labor spent in stripping 1.16 pounds of fat were secured.

Massaging the udder during two minutes of the time the machine was operating decreased the amount of strippings 33 per cent. Manipulation (pulling down) of the teat cups for one minute caused a 55 per cent reduction in the amount of strippings. The cows were milked more thoroughly by the machine in five minutes if manipulation of the teat cups was practiced for one minute (the last) than in six minutes of normal machine milking.

REFERENCES

- (1) M'CANDLISH, A. A., AND COCHRANE, JAMES. Milking machines. *Scottish Jour. of Agr.*, 13: 43-50. 1930.
- (2) MATTHEWS, C. A., SHAW, J. M., AND WEAVER, EARL. The economy and efficiency of a milking machine. *Iowa Agr. Exp. Sta. Bul.* 248. 1928.
- (3) PETERSEN, W. E., AND SWENSON, PHILIP. A method for studying the question of stripping following machine milking. Abstracts of papers presented at the 27th annual meeting of the American Dairy Science Association, p. 5. 1932.
- (4) RAGSDALE, A. C., TURNER, C. W., AND BRODY, S. The rate of milk secretion as affected by an accumulation of milk in the mammary gland. *JOUR. DAIRY SCIENCE* 7: 249-254. 1924.
- (5) WALLIS, G. C., ECKLES, C. H., PETERSEN, W. E., AND MACY, H. The influence of non-stripping on the bacterial content of machine-drawn milk. *JOUR. DAIRY SCIENCE* 15: 163-171. 1932.
- (6) WOLL, F. W., AND HUMPHREY, G. C. The efficiency, economy and physiological effect of machine milking. *Wisc. Agr. Exp. Sta. Res. Bul.* 3. 1908.
- (7) WOODWARD, T. E. The effect of leaving milk in the udder at each milking on persistency of yield. Abstracts of papers presented at the 27th annual meeting of the American Dairy Science Association, p. 60. 1932.

American Dairy Science Association Announcements

ANNUAL MEETING

Ithaca and Geneva, New York, June 26 to 28, 1931

It is expected that many members will again combine their summer vacation and attendance at the annual meeting. Entertainment is being planned for the women and children. There are many points of scenic and historic interest in this vicinity. There will be a registration and information desk in the Dairy Industry Building in Ithaca on June 25 and 26.

Members are urged to promptly send in titles of papers to J. M. Sherman, Ithaca, N. Y., as it is hoped that the program will be completed in time to appear in the June number of the JOURNAL OF DAIRY SCIENCE.

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THE UTILIZATION OF ATLAS AND KANSAS ORANGE SORGO SEED BY DAIRY COWS¹

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The increasing popularity of Atlas sorgo both as a grain and a silage crop has caused many dairymen to inquire regarding methods of handling this crop for dairy cattle. In the sections of the southwest where Kansas Orange sorgo is grown this crop is generally compared with Atlas sorgo. Atlas differs from Kansas Orange chiefly in being more resistant to lodging and in having white palatable grain, whereas the grain of Kansas Orange is brown and somewhat bitter. Also, the heads of Atlas appear to make up a greater portion of the total weight of the plant than do those of some other sorgos and for this reason many users are reluctant about putting Atlas sorgo heads into the silo. It was to obtain data on the utilization of the grain in these two crops that this experiment was planned.

Review of Literature

Experimental work at this station (1) has indicated that Kansas Orange silage and Atlas silage are practically equal as a feed for dairy cattle. Early work at the same station demonstrated that sorghum silage was only slightly less valuable than corn silage (2) in spite of the fact that a large number of the sorghum grains pass through the cows undigested. The loss of grain in the droppings of cows fed sorghum silage was first studied by Reed and Fitch (3). By washing out the droppings of several cows fed corn silage, Sumac sorgo silage, and kafir silage it was estimated that about 4 per cent of corn grain, 30 per cent of kafir grain, and 90 per cent of the Sumac sorgo seed passed through the cows undigested.

Becker and Gallup (6) found by feeding cane silage and kafir silage to dairy cows that 33.91 per cent of the cane seeds were lost and 49.46 per cent of the kafir seeds were lost. It was also found that a small per cent of the nutritive value was removed from the whole grain as it passed through the digestive tract.

Aicher and McCampbell (4) found that kafir silage with the heads had a slightly higher feeding value for steers than cane silage with the heads,

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¹ Contribution Number 91 from the Department of Dairy Husbandry.

a difference of approximately three per cent. When feeding the silage without the heads, the cane silage was superior to the kafir silage in feeding value by 15 per cent.

Thompson (5)* found that grinding of kafir seed for hogs increased the feeding value 10 to 25 per cent over the whole grain.

This station (7) found that the feeding value of ground kafir seed and ground cane seed compared very favorably to corn when fed to dairy cows.

EXPERIMENT I

Plan of Experiment

1. Three cows were used in the experiment—one Holstein 10 years of age, one Holstein 3 years of age, and one Ayrshire 8 years of age. The Ayrshire was dry and the two Holsteins were in the later stages of lactation. The mouths of all the cows were examined by Dr. E. R. Frank of the Veterinary Division and all were pronounced sound.

2. The cows were fed individually according to size and milk production and all three received the same ration but in varying amounts. They were fed liberally and received all the feed they would clean up. When the cows were receiving silage of either Atlas or Kansas Orange, they received a grain mixture of cracked corn, ground oats, wheat bran, and cottonseed meal. When the cows received the whole seed of Kansas Orange or Atlas as the sole concentrate, only alfalfa hay was fed with it.

3. Each experimental period comprised four days of preliminary feeding and a five day period of collection. In the first phase of the experiment the cows received Kansas Orange silage, alfalfa hay, and the grain mixture. The second phase was the same except that Atlas silage was substituted for the Kansas Orange silage. In the third phase of the experiment the cows received Kansas Orange seed, unground, along with alfalfa hay. In the fourth phase Atlas seed was substituted for the Kansas Orange seed.

4. The Kansas Orange silage was taken about 15 feet down in a 30 foot silo. The Atlas silage was taken about 10 feet down in a 30 foot silo. Both crops were mature before ensiling.

5. The cows were kept in stalls throughout the experiment. The manure from each cow was collected and weighed. One-tenth of the manure from each cow was washed separately on a one-fourteenth inch mesh sieve to determine the amount of seeds lost in the feces.

6. The total amount of seed in the silage was determined on a five pound sample. This was air dried and the seeds removed by hand.

7. The seeds recovered in the manure were air dried to put them on the same basis as the seeds from the silage mentioned above.

8. In the third and fourth phases the whole grain was air dried so as to give results comparable to the first two phases.

9. Seeds removed from the silage and manure were saved for germination tests, as were also the whole grain before feeding and the grain recov-

ered in the manure. The germination tests were made by the State Seed Laboratory.

10. Chemical analyses were made of the seeds from the silage, whole grains, and the seeds recovered in manure from each phase of the experiment.

Results of Experiment

On the air dry basis the Kansas Orange silage contained 68.4 per cent moisture and the Atlas 60.6 per cent. The Kansas Orange silage contained 27.7 per cent seeds, by air dry weight, which represented 8.76 per cent of the fresh silage. The Atlas silage contained 25.92 per cent seeds on the air dry basis which represented 9.79 per cent of the fresh silage. The whole grain fed, contained 95.31 per cent dry matter on the air dry basis. Both grains showed the same per cent of moisture. The data on the individual cows are presented in table 1.

TABLE 1

*Losses of grain in Kansas Orange and Atlas silage when fed to dairy cows
Five day Kansas Orange period*

COWS	SILAGE CONSUMED	GRAIN IN SILAGE	AMOUNT OF GRAIN IN SILAGE AIR DRY BASIS	AMOUNT OF SEED RE- COVERED IN FECES	GRAIN RECOVERED IN FECES
	(pounds)	(per cent)	(pounds)	(pounds)	(per cent)
279	262.5	8.76	23.00	7.20	31.3
160	212.5	8.76	18.62	7.78	41.8
195	262.5	8.76	23.00	12.78	55.6
Total	737.5		64.62	27.76	42.9 (Av)

Five day Atlas period

279	187.5	9.79	18.36	5.62	30.61
160	150.0	9.79	14.68	3.75	25.54
195	235.0	9.79	23.01	10.94	47.54
Total	572.5		56.05	20.31	36.24 (Av)

The results of feeding whole grains with alfalfa hay are given in table 2.

Both tables 1 and 2 show that the per cent of loss was greater with Kansas Orange seed than with Atlas seed. The loss was considerably larger when the grain was fed as the sole concentrate than when fed in the form of silage. This difference may be attributed to the softening of the seeds in the process of ensiling.

Table 3 gives a brief summary of the seed losses and also includes the per cent germination of seeds from all phases of the experiment.

In this experiment the ensiling process apparently destroyed the germination of all Kansas sorgo seed and Atlas seed.

In order to determine whether any nutritive value had been removed from the whole seeds while passing through the digestive tract chemical analyses were made of all samples of seed from the four phases of the ex-

TABLE 2

Losses of grain when Kansas Orange seed and Atlas seed were fed unground as the only concentrate

Five day Kansas Orange period

COWS	GRAIN FED	DRY MATTER IN GRAIN AIR DRY BASIS	GRAIN FED AIR DRY BASIS	GRAIN RECOVERED IN FECES	GRAIN RECOVERED IN FECES
	(pounds)	(per cent)	(pounds)	(pounds)	(per cent)
279	27.0	95.31	25 73	15.31	59.50
160	24.5	95.31	23 35	13.84	59.27
195	54.0	95.31	51.47	33.60	65.28
Total	105.5		100.55	62 75	62.41 (Av)

Five day Atlas period

279	22 5	95.31	21 44	11.56	53.92
160	22.5	95.31	21 44	13.00	60.63
195	37.5	95.31	35.74	15.34	42.92
Total	82.5		78.62	39.90	50.75 (Av)

TABLE 3

Summary of seed losses and germination tests

FEEDS	SILAGE (CON- SUMED)	SEEDS IN SILAGE	WEIGHT OF SEEDS CONSUMED	WEIGHT OF SEEDS RECOVERED IN FECES	SEEDS WASTED	GERMI- NATION BEFORE FEEDING	GERMI- NATION AFTER FEEDING
	(pounds)	per cent	(pounds)	(pounds)	per cent	per cent	per cent
Kansas Orange silage	(3 cows) (5 days) 737.5	8.76	64.62	27.76	42.95	0	0
Atlas silage	572.5	9.79	56.05	20.31	36.24	0	0
Kansas Orange whole grain as the only concentrate			100.55	62.75	62.41	95	11
Atlas sorgo whole grain as the only concentrate			78.62	39.90	50.75	94	6

periment by the Chemistry Department. The results of these analyses are shown in table 4.

Consideration of the results in table 4 indicates that the digestive tract of the bovine removes little of the food value from the whole seeds whether consumed in the form of silage or as a concentrate. Approximately 20

TABLE 4
*Percentage composition of Kansas Orange and Atlas seed
from the four phases of the experiment
(Air dry basis)*

MATERIAL	MOISTURE	DIGESTIBLE CRUDE PROTEIN	ETHER EXTRACT	CRUDE FIBER	ASH	N-FREE EXTRACT
Kansas-Orange seed from silage	6.54	7.88	3.83	2.53	1.28	77.94
Kansas Orange seed recovered from feces	7.28	7.69	2.98	2.49	1.01	78.55
Atlas seed from silage	6.07	10.19	3.85	2.09	1.24	76.56
Atlas seed from feces	7.10	10.94	3.28	1.69	0.80	76.21
Whole Kansas Orange grain before feed- ing	7.35	10.94	3.21	1.74	1.21	75.55
Whole Kansas Orange grain from feces	6.81	10.81	2.88	2.20	1.30	76.00
Whole Atlas grain before feeding	7.19	9.38	3.70	1.44	1.50	76.79
Whole Atlas grain from feces	6.52	8.81	3.03	1.49	0.82	79.33

per cent of the ether extract and 30 per cent of the ash was removed in the above samples. These constituents make up only a small per cent of the total food value. The increase in ash content of the Kansas Orange seeds when fed whole as the only concentrate may have been due to the presence of some foreign material in the sample.

EXPERIMENT II

Plan of Experiment

A second experiment was conducted with Atlas sorgo silage in connection with a digestion trial which was being run in the experimental barn. Eight cows representing the four major dairy breeds were used. Information concerning the cows used in this experiment is given in table 5.

The ration fed consisted only of Atlas sorgo silage. The amount of silage fed each cow was determined by the animal's appetite but remained constant throughout the period of collection. All silage fed was weighed and the weights rechecked by a second person. The trial covered a period of 20 days of which the first 10 days constituted the preliminary period,

TABLE 5
Cows used in the second experiment

NUMBER	BREED	AGE	STAGE OF LACTATION	STATE OF GESTATION
		(years)		(months)
1	Ayrshire	4	Dry	5
2	Guernsey	11	"	5
3	Holstein	4	"	7
4	Guernsey	6	"	5
5	Jersey	8	"	Open
6	Ayrshire	4	"	5
7	Jersey	5	"	5
8	Ayrshire	6	"	5

while the second 10 days were used for collecting the data. The silage fed was similar to that used in the previous trial and was from the same field and silo but was taken about 12 feet lower in the silo. A 100 gram composite sample of silage was taken each day for moisture determination and percentage of seeds. After separation of the seeds the whole sample was put in an oven to dry at 80° C.

As this trial was conducted in connection with a digestion trial, an attendant was present to catch all the feces. The feces were weighed and re-checked by a second person. The twenty-four hour composite for each cow was thoroughly mixed and ten per cent was saved. A portion was taken from each daily composite to make a ten day composite. At the close of the trial this composite was thoroughly mixed and twenty per cent saved for determination of seeds in the feces. As in the previous experiment the feces were washed on a one-fourteenth inch mesh screen. The seeds recovered were dried for 5 days in an oven at 80° C.

Results of Experiment

The Atlas silage contained 67.13 per cent moisture. On the dry matter basis the seeds constituted 28.04 per cent of the silage. On the fresh silage basis the seeds represented 8.28 per cent of the silage. The data from the second experiment are given in table 6.

The unusually low percentage of loss for the Holstein cow No. 3 can, perhaps, be explained by the fact she had previously been fed on a similar

ration and had become accustomed to it. If she were left out of the calculation, the average per cent loss would be 33.00 instead of 30.39.

It appears feasible, however, to expect a lower percentage of loss in this trial than the previous one because only silage was fed. The first trial, however, was conducted under more common conditions of feeding which would have a bearing on the practical value of the results obtained.

TABLE 6

Losses of grain when Atlas sorgo silage was fed to dairy cows

COW NUMBER	BREED	SILAGE CONSUMED	DRY SEED CONSUMED	SEED RECOVERED	SEED RECOVERED IN FECES
		(grams)	(grams)	(grams)	(per cent)
1	Ayrshire	199760	16540	6700	40.51
2	Guernsey	108960	9022	2850	31.59
3	Holstein	172520	14285	2000	14.00
4	Guernsey	108960	9022	1825	20.23
5	Jersey	163440	13533	3450	25.49
6	Ayrshire	172520	14285	4350	30.45
7	Jersey	145280	12029	3800	31.59
8	Ayrshire	181600	15036	6550	43.56
Total		1253040	103752	31525	30.39 (Av)

CONCLUSIONS

1. Approximately 43 per cent of the seed in Kansas Orange sorgo silage and 36 per cent of the seed in Atlas sorgo silage were voided in the feces of dairy cows when fed in the dairy ration with alfalfa hay and a grain mixture; when fed alone, in the form of silage, 30 per cent of the seeds in Atlas silage were lost. When the two grains were fed as the sole concentrate with alfalfa hay, 62 per cent of the Kansas Orange seed and 51 per cent of the Atlas sorgo seed were voided in the feces.

2. In the experiments reported 19 per cent more of the Kansas Orange seeds and 15 per cent more of the Atlas seeds were utilized by the animals when silage was fed than when the whole grain was fed.

3. The animals used a negligible amount of the food nutrients from the whole seeds recovered in the feces. In this experiment the ensiling process destroyed the germination of the Kansas Orange and Atlas seeds. When the seeds were fed without being ensiled, the digestive tract greatly reduced the germination of the whole seeds which were voided in the feces.

REFERENCES

- (1) CAVE, H. W., AND FITCH, J. B. Ground Sorgo Seed as a Feed For Dairy Cows. Kansas Agric. Exp. Sta. Circular 110. 1925.

- (2) REED, O. E., AND FITCH, J. B. Sorghum Crops For Silage. Kansas Agric. Exp. Station Circular 28. 1913.
- (3) Unpublished data (1913-1914).
- (4) AICHER, L. C., AND McCAMPBELL, C. W. Pamphlet. 1926.
- (5) THOMPSON, C. P. Oklahoma Feeds and How to Prepare Them. Okla. Agric. Exp. Sta. Bulletin 148.
- (6) BECKER, R. B., AND GALLUP, WILLIS D. Utilization of the Grain in Kafir and Cane Silage by Dairy Cows. Okla. Agric. Exp. Sta. Bulletin 164. 1927.
- (7) CAVE, H. W., AND FITCH, J. B. Ground Kafir as a Feed For Dairy Cows. Kansas Agric. Exp. Sta. Circular 119. 1925.

STUDIES OF THE TECHNIC TO EVALUATE THE EFFICIENCY OF SEVERAL CHLORINE STERILIZERS FOR DAIRY USE*

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LITERATURE REVIEW

Although considerable work has been done on the value of chlorine as a sterilizer on the dairy farm and in the creamery and the use of chlorine has become a well established practice, new methods of examination warrant another investigation of chlorine as a disinfectant and a critical study of the various types of chlorine preparations on the market.

In connection with a study of the disinfection of swimming pool water, Mallmann and Cary (1) found that samples from chlorinated pool water when tested immediately after collection showed considerably more pollution than duplicate samples tested at the laboratory several hours later. It is quite obvious, however, that the residual chlorine in the sample would, if the sample was held for a period of time, cause a gradual disinfection with the ultimate destruction of the bacteria present. A similar condition would exist in samples taken of chlorine rinse water used in the sterilization of dairy pipe lines, except that a more pronounced condition would exist. As far as the writers could determine, in all studies purporting to check the efficiency of chlorine rinse waters the action of the residual chlorine was not checked at the time of sampling. In the studies herein presented, a comparison of the usual method of sampling and a method wherein the residual chlorine was removed at the time of collection was employed.

Mallmann and Cary suggest the use of a sodium thiosulphate treated sample bottle for collecting samples containing residual chlorine for bacteriological analysis. The addition of the sodium thiosulphate to the sample bottle inactivates the residual chlorine as soon as the sample enters the bottle, and thus prevents the destruction of the bacteria while the sample awaits analysis.

That hypochlorites do not have the same oxidizing power at varying pH values was first demonstrated by Rideal and Evans (2) when they showed that the additions of alkali caused a depression of the oxidizing power of the hypochlorites. That a reduced bactericidal activity was also produced by the addition of alkali to hypochlorites in dilute solutions using short periods of exposure was demonstrated by Mallmann and Schalm (3).

A study was made by Johns (4) of the speed of destruction of mixed

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organisms from milk cans. The action of three hypochlorites and two chloramine T products was tried on *Escherichia coli*, *Aerobacter aerogenes*, *Streptococcus lactis*, and spores of *Bacillus subtilis*. The liquid hypochlorites were effective against all non-spore formers with the exception of a highly alkaline hypochlorites which acted surprisingly slow against *S. lactis*. The chloramine T products in all cases acted too slowly to be considered suitable as sterilizing rinses. None of the five products had any noticeable effect upon the spores of *B. subtilis* during a 2 minute exposure. It was also noted by Johns (5) that the less alkaline hypochlorites were extremely effective against five rosy and two bitter milk organisms. Prucha (6) demonstrated that the chloramine T compound which he used displayed a delayed germicidal action against *Esch. coli* when comparisons were made to the hypochlorites. *Strept. lactis* offered considerable resistance to both types of compounds. In connection with a study of the disinfection of swimming pool water Mallmann and Cary (1) also noted a delayed germicidal action of chloramine treatment.

EXPERIMENTAL

Three commercial chlorine sterilizers were studied—"A" was a sodium hypochlorite compound containing trisodium phosphate, while "C" was a sodium hypochlorite and sodium carbonate mixture. Sterilizer "B" was a chloramine T preparation containing sodium carbonate. These compounds were employed under commercial conditions in the college creamery which may be contrasted to the work of Johns (4) in which the work was conducted largely in the laboratory. Although the work of Prucha (6) was done both under laboratory and commercial conditions the data presented were not of as strictly a quantitative nature as the data to be presented here. The equipment was washed in the usual manner without dismantling on Sundays, Mondays, Wednesdays, Thursdays, Saturdays and chlorinated the following mornings prior to use. On Tuesdays and Fridays after use the equipment was dismantled and cleaned in the customary manner while in this condition. The chlorination process consisted in pumping approximately 75 gallons of the sterilizer from the receiving vat, in which it was prepared, to the pasteurizer, where it was splashed around in order to bring it in contact with all surfaces. From the pasteurizer it was allowed to flow over the cooler and through the bottle filler. One hundred cc. samples for chemical determinations were taken of the stock solution at the receiving vat and samples for chemical and bacteriological determinations were taken at the bottle filler. Those designated as "start of chlorine rinse" consisted of the first of each solution to reach the bottle filler at the beginning of each chlorine rinsing process. Those designated as "middle of chlorine rinse" were taken at the bottle filler after about 35 to 40 gallons had passed through the equipment. Those samples taken when last of the rinse was

about to leave the bottle filler were designated as "end of chlorine rinse." About seven minutes' time was required for the entire 75 gallons of chlorine rinse solution to pass through the equipment. At each time of sampling at the bottle filler one sample was taken into an empty sterile bottle and one into a sterile bottle containing approximately 0.1 gram of sodium thiosulphate, as recommended by Mallmann and Cary (1). This was added in crystal form to these sample bottles prior to autoclaving¹ and was sufficient to inactivate all the chlorine in the rinse so that there would be no further destruction of bacteria present. Three trials were made on each of three different concentrations of the sterilizers, and six trials were made on a fourth concentration of sterilizer "C." Approximately one hour after chlorination and immediately prior to use the equipment was rinsed for about ten minutes by pumping tap water through it. Samples of this rinse water were taken at the start, middle and end of this run into sterile bottles containing no thiosulphate.

The titration figures given in table 1 for the stock solutions and bottle filler samples of the chlorine and rinse waters are the averages for three trials on each concentration of each sterilizer. In like manner the average bacterial plate counts are given on all of the bottle filler samples in table 2. The ortho-tolidine and iodometric methods were employed for determining quantitatively the p.p.m. of available chlorine.

At the time the "middle of the chlorine rinse" samples were taken for chemical and bacteriological examination 500 cc. samples were taken to determine the germicidal powers of the various rinse solutions, using the technic of Mallmann and Schalm (3). To these were added a 24-hour culture of *Esch. coli* and after exposure to the chlorine for 15, 30, 45, 60, 90, 120, and 180 seconds the presence of surviving organisms was determined qualitatively and quantitatively. To do this one cc. of material was drawn from the flask at these time intervals and inoculated into 9 cc. tubes of plain broth, from which plates were made from proper dilutions immediately after the last sample was removed. The broth not only served as a means of immediately checking any further action of the chlorine but also, when incubated, served as a means for detecting qualitatively the presence of living organisms. The average counts for three trials on each concentration of each of the sterilizers are given in table 3 with the results of the broth tubes.

DISCUSSION

The two methods employed for measuring quantitatively the amount of available chlorine gave approximately the same results in all cases (Table 1). The fact that the ortho-tolidine test is colorimetric and could not be read

¹ Recently it has been found advisable to add the thiosulphate in dry form to a dry bottle and sterilize in dry heat at a temperature not to exceed 200° C.

TABLE 1
Concentrations of the chlorine rinse solutions and rinse waters determined by the ortho-tolidine and iodometric methods

STERILIZER	A						B						C											
	AVERAGES OF 3 TRIALS ON EACH CONCENTRATION						AVERAGES OF 3 TRIALS ON EACH CONCENTRATION						AVERAGES OF 3 TRIALS ON EACH CONCENTRATION											
	p.p.m.		p.p.m.		p.p.m.		p.p.m.		p.p.m.		p.p.m.		p.p.m.		p.p.m.		p.p.m.		p.p.m.		p.p.m.		p.p.m.	
	O.T.	I.	O.T.	I.	O.T.	I.	O.T.	I.	O.T.	I.	O.T.	I.	O.T.	I.	O.T.	I.	O.T.	I.	O.T.	I.	O.T.	I.	O.T.	I.*
Method of titration	77	78	43	45	15	19	67	85	72	53	60	26	36	60	79	42	43	15	17	6	11			
Stock solution																								
Start of chlorine rinse (n.t.)	60	69	30	29	10	12	60	72	53	58	23	23	32	53	58	20	26	7	13	1	4			
Middle of chlorine rinse (n.t.)	77	80	40	39	13	17	63	79	53	60	23	23	35	53	68	33	38	13	17	3	7			
End of chlorine rinse (n.t.)	77	76	40	39	13	17	65	78	50	60	23	23	35	53	69	38	38	13	17	3	6			
Start of chlorine rinse (t.)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			
Middle of chlorine rinse (t.)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			
End of chlorine rinse (t.)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			
Start of water rinse	0.7	0.5			0.2		1.5		0.2	0.2	0.1			0.3		0.2		trace		trace				
Middle of water rinse	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			
End of water rinse	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			

n.t. = no sodium thiosulphate in the sample bottle.

t. = sodium thiosulphate in the sample bottle.

O.T. = ortho-tolidine determination.

I. = iodometric determination.

* = averages of 6 trials.

accurately within 10 p.p.m. in the concentrations employed accounts for the majority of the differences. The table also displays the fact that the chlorine content in the first material to pass through the equipment was somewhat lowered, due undoubtedly to combination with organic materials. However, the concentration at the end of the chlorination process approached that of the stock solution. In all cases the sodium thiosulphate completely inactivated the chlorine in the samples taken in bottles containing this compound. This made it possible to obtain the actual bacterial content of the rinse solutions, although they were not plated until several hours had elapsed. At the start of the rinsing of the equipment with tap water, about an hour after chlorination, there was invariably some residual chlorine present, the quantity varying from a trace to 2.0 p.p.m.

In table 2 the value of the sodium thiosulphate sample bottle is clearly shown when comparisons are made of counts made from the sample bottles containing no thiosulphate to check further action of the chlorine and those counts made from sample bottles containing the thiosulphate. As judged by the former set of counts the equipment would have been considered very clean in all cases, while the counts made from the thiosulphate sample bottles afforded a means of differentiating clean from unclean equipment. In this respect the two inorganic hypochlorite sterilizers (A and C) apparently acted more efficiently under the conditions of the experiment. This was undoubtedly due to the more available condition of the chlorine. The counts made on the rinse water samples were in keeping with the final counts made on the thiosulphate treated chlorine samples.

The data on the disinfecting powers of the various chlorine solutions after having been used as such are given in table 3. These solutions, as mentioned before, were taken at the bottle filler after 35 to 40 gallons of the chlorine solution had passed through the equipment. Sterilizers "A" and "C" were again shown to be very efficient bacteriologically in relatively low concentrations when the latter were measured by chemical methods, while sterilizer "B" displayed a delayed germicidal action. The value of a bacteriological test of this type is very readily seen, especially when one bears in mind the fact that the chemical titrations on these rinse solutions, after having been used as such, do not show such bactericidal differences to exist. In the creamery these rinse solutions exist in an alkaline condition which has been shown to reduce the oxidizing or disinfecting powers (2) (3) of these compounds. However, when these same solutions were titrated according to accepted methods the titrations were done in an acidified medium, a condition in which the chlorine becomes a very active disinfecting agent. As a result a figure is obtained which would indicate a relatively high disinfecting value of the rinse solution as it exists in the creamery, which, according to the bacteriological test, is shown actually not always to exist. The inaccuracy of chemical tests for measuring germicidal

TABLE 2
Bacterial counts on the chlorine rinse solutions and the rinse waters. Averages of three trials on each concentration of chlorine

STERILIZER	A						B						C					
	O.T.	77	O.T.	43	O.T.	15	O.T.	67	O.T.	53	O.T.	26	O.T.	42	O.T.	15	O.T.	6
	I	78	I	45	I	19	I	85	I	60	I	36	I	43	I	17	I	11
ppm *	count per cc.		count per cc.		count per cc.		count per cc.		count per cc.		count per cc.		count per cc.		count per cc.		count** per cc.	
Start of chlorine rinse (n.t.)	0	1	0	12	7	42	1	0	0	0	0	0	0	0	0	0	4	4
Middle of chlorine rinse (n.t.)	0	1	0	4	2	2	0	0	0	0	0	0	0	0	0	0	5	5
End of chlorine rinse (n.t.)	0	0	0	4	1	1	0	0	0	0	0	0	0	0	0	0	5	5
Start of chlorine rinse (t.)	28	26	51	7900	283	2766	38	166	11	262	11	262	11	262	11	262	11	262
Middle of chlorine rinse (t.)	2	11	12	27	9	295	114	16	139	72	16	139	72	16	139	72	16	139
End of chlorine rinse (t.)	9	53	3	11	32	7	0	55	235	128	55	235	128	55	235	128	55	235
Start of water rinse	1	2	10	9	7	273	0	0	0	0	0	0	0	0	0	0	19	19
Middle of water rinse	2	3	11	12	0	9	16	42	52	88	42	52	88	42	52	88	42	52
End of water rinse	13	2	4	21	0	11	19	15	44	6	15	44	6	15	44	6	15	44

* = averages of three trials on each concentration (see table 1).

O.T. = ortho-tolidine.

I. = iodometric.

n.t. = no sodium thiosulphate in the sample bottle.

t. = sodium thiosulphate in the sample bottle

** = averages of six trials.

TABLE 3
Disinfecting powers of the various chlorine solutions after having been used as such. Averages of three determinations on each concentration.
Test organism—24 hour culture of Esch. coli

STERILIZER	A				B									
	O.T.	83	40	13	70	50	22							
	I.	81	40	17	76	60	36							
Concentration—p.p.m.	Count per cc.		Count per cc.	Broth	Count per cc.		Broth							
	Broth		Broth		Broth		Broth							
No. of seconds contact	15	0	±	0	3,100	±	±	±	55,136	+	77,300	+	173,000	+
	30	0	—	0	2,300	±	±	±	29,385	+	64,700	+	170,300	+
	45	0	—	0	0	—	—	—	11,526	+	52,000	+	112,000	+
	60	0	—	0	0	—	—	—	7,528	+	39,000	+	105,300	+
	90	0	—	0	0	—	—	—	6,268	+	28,200	+	76,600	+
	120	0	—	0	0	—	—	—	617	+	34,200	+	86,200	+
	180	0	—	0	0	—	—	—	0	±	6,700	+	51,500	+
	Control	750,000		205,300	141,000	2,324,000	156,300	302,000						

TABLE 3—(Continued)

STERILIZER	C							
	O.T. 53		30		13		4	
	Count per cc.	Broth	Count per cc.	Broth	Count per cc.	Broth	Count per cc.	Broth
Average chlorine Concentration—p.p.m.	I. 72		36		17		7	
No. of seconds contact								
15	538	+	55	±	0	-	360	±
30	0	±	0	-	0	-	0	±
45	30	±	0	±	0	-	0	-
60	0	-	0	-	0	-	0	-
90	0	±	0	-	0	-	0	-
120	0	-	0	-	0	-	0	-
180	0	-	0	-	0	-	0	-
Control	363,000		298,000		94,000		258,000	

properties of such solutions is probably increased at times, since some of the chlorine which enters the titrations is undoubtedly in an adsorbed condition making it non-effective as a disinfecting agent.

In laboratory studies, in which actual working conditions are supposedly simulated, it is frequently true that this is not the case. Mallmann and Edwards (7) point out that statements of chlorine concentration do not necessarily present a true state of field conditions. For example, they cite an instance where 55 p.p.m. available chlorine was used and another where 0.6 p.p.m. was used. In the instance where 0.6 p.p.m. was used, more total chlorine was present. In actual practice a rinse of 50 gallons of 10 p.p.m. chlorine content would be superior to 10 gallons of 50 p.p.m. chlorine, although the total chlorine content of each is exactly the same. The weaker solution would be more efficient because of the longer time period necessary to pass the rinse through the equipment. In the data presented, it was found that 6 to 11 p.p.m. of hypochlorite "C" gave as efficient results as 60 to 79 p.p.m. in checks on the rinse solution as it passed through the pipe lines and further the rinse solution when tested subsequently for germicidal activity gave very similar results. In other words under the conditions of the test, 75 gallons of a 6 to 11 p.p.m. chlorine rinse had sufficient chlorine to destroy all bacteria in the equipment and still have an excess activity after use to kill large numbers of *Esch. coli* under laboratory conditions. Thus under the conditions of the experiment the chlorine rinse had at least an excess safety factor of 54 to 68 p.p.m. chlorine. This would seem to indicate that the present standard of 50 p.p.m. chlorine would be ample on all types of equipment, providing of course that the volume used was proportionate to that used in these experiments. To place more emphasis on the time factor it could be recommended that a large enough quantity of rinse solution be used which would require a minimum time period of 5 minutes to flow through the equipment. In this event there would undoubtedly be cases where much less than 50 p.p.m. chlorine would be sufficient. Inspectors checking chlorine residuals of rinse waters should be equally interested in knowing volumes of rinse solution used as the latter is even more important than the actual concentration.

While it is a well known fact that the chloramine T compounds display a delayed germicidal action, they are not to be considered entirely unsuitable for dairy use, as has been done by some investigators. Use can be made of these compounds when it is unnecessary to obtain immediate sterilization as, for example, when it is the practice to sterilize the equipment an hour before using it. This intervening time period gives the chloramine T compounds sufficient time to bring about destruction of microorganisms, as is shown by table 2, in which it is noted that the water rinse, made approximately an hour after the chlorine rinse, was found to contain the same relatively small numbers of organisms regardless of the type of chlorine solution previously used.

SUMMARY

Sterile sample bottles containing sodium thiosulphate were found more suitable than the usual sample bottle for obtaining samples of chlorinated dairy solutions intended for bacteriological examination.

The iodometric and ortho-tolidine tests do not necessarily measure the germicidal powers of used chlorine sterilizers as measured by the actual conditions evidenced by bacteriological tests.

In actual practice using the sodium thiosulphate treated sample bottle the hypochlorite sterilizers were found to be effective against the organisms in the dairy equipment. The used solutions in all concentrations were found to display a marked germicidal action against *Esch. coli* when tested in the laboratory.

The chloramine T compound as used in these experiments displayed a delayed germicidal action which might make it objectionable as a dairy sterilizer under certain conditions.

Hypochlorite rinse solutions with residual chlorine contents as low as 6 p.p.m. were found effective in the disinfection of dairy equipment. In reference to hypochlorites, any chlorine in excess of 6 p.p.m. constituted a safety margin.

The use of large volumes of rinse is more important than high chlorine residuals. This is particularly important in the use of chloramine T compounds which require a longer period of exposure to effect sterilization.

REFERENCES

- (1) MALLMANN, W. L., AND CARY, WILLIAM. Study of bacteriological methods of testing and means of disinfecting water with chlorine. *Amer. Jour. Pub. Health* 23: 35-44. 1933.
- (2) RIDEAL, E. K., AND EVANS, V. P. The effect of alkalinity on the use of hypochlorites. *Jour. Soc. Chem. Ind.* 40: 64R-66R. 1921.
- (3) MALLMANN, W. L., AND SCHALM, OSCAR. The influence of the hydroxyl ion on the germicidal action of chlorine in dilute solutions. *Mich. Eng. Exp. Sta. Bull.* 44. 1932.
- (4) JOHNS, C. K. The speed of germicidal action of chlorine compounds upon bacteria commonly occurring in milk. *Sci. Agr.* 10: 553-563. 1930.
- (5) JOHNS, C. K. The destruction of rosy and bitter milk organisms by hypochlorite. *Sci. Agr.* 12: 38-42. 1931.
- (6) PRUCHA, M. J. Chemicals are effective sterilizers. *Ice Cream Review*. Feb. 1928. Also from a paper delivered at the Annual Meeting of the Amer. Pub. Health Assn., Indianapolis, Oct. 1933.
- (7) MALLMANN, W. L., AND EDWARDS, O. F. The germicidal activity of available chlorine as measured by the ortho-tolidine and iodometric tests for chlorine. Submitted for publication 1933.

PIMIENTOS IN PROCESSED CHEESE

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Some time ago the authors were requested to make a study of the use of pimientos in processed cheese in an effort to determine why certain varieties or sources of supply received preference to the practical exclusion of other types that were available. In view of the dearth of information on pimientos and their use in processed cheese, the results of this brief survey are presented in this paper.

Standard brands of canned pimientos were supplied in sufficient quantity to permit the manufacture of one or more batches of processed cheese. The brand that did not receive preference from the commercial processor was supplied in largest quantity in order that it might be studied in greater detail. The only reason that could be given as to why this pepper was not used was that it had been reported as giving the cheese a slightly sour taste.¹

The pimientos were classified according to source, one brand from Georgia, one from California and two from Spain. The cans of each brand were opened separately and the content examined for color, aroma and hydrogen-ion concentration. These observations are presented in table 1.

The general appearance of 3 was very good as all the pieces were very uniform in color. The hydrogen-ion determinations were made with the hydrogen electrode on the liquid that was drained from the peppers. The color of this liquid did not permit the determination of the titratable acidity with any degree of accuracy. The variations of the hydrogen-ion concentration are in very good agreement with those obtained by other workers on the same types of pimientos.¹

The cheese used in these experiments was a blend of fairly good quality, the average age being about six months. To determine the acidity of the cheese fifty grams of the comminuted cheese were suspended in 500 cc. of distilled water with vigorous agitation. Twenty-five cc. portions of this suspension were then used for the determination of the titratable acidity, which was found to be 1.82 calculated as lactic acid. Hydrogen-ion determinations made on this suspension gave a pH value of 5.68. The processing was performed in the cheese kettle described previously,² using six or

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¹ FELLERS, C. R., Massachusetts State College. Private correspondence.

² TEMPLETON, HUGH L., AND SOMMER, H. H. Some observations on processed cheese. *JOUR. DAIRY SCI.* 13: 203. 1930.

TABLE 1
Pimientos in processed cheese

CHEESE SAMPLE No.	PIMIENTOS USED			PIMIENTO CHEESE		
	Brand No.	Color of pimientos	Aroma	pH	Moisture %	Titratable Acid %
1	1	light red and not uniform	very slight	4.55	38.5	0.95
2	1	" " " "	" "	4.55	39.2	1.23
3	1	" " " "	" "	4.55	39.7	1.11
4	1	" " " "	" "	4.55	40.0	1.13
5	1	" " " "	" "	4.55	41.0	0.95
6	2	not uniform red	good	4.59	41.5	1.01
7	2	" " " "	" "	4.59	41.5	1.01
8	3	uniform dark red	very pungent	4.84	39.5	0.97
9	4	not uniform red	good	4.71	41.7	1.07
10	2 (83.5%) 4 (16.5%)				41.2	0.95

Additions to samples: 3, 2.8 gms. sodium bicarbonate.
 4, 1.4 " " "
 5, 70.0 " " whey powder.
 7, 1.0 " " citric acid.
 9, 1.0 " " sodium bicarbonate.
 10, 2.0 " " citric acid.

seven pounds of cheese to a batch, depending upon the amount of pimientos available. This amount of cheese was necessary to insure the proper working of the thermometer in the kettle. The weight of pimientos added represented 8.0 per cent of the total weight in the kettle. No effort was made to comminute the peppers before addition to the cheese, as the action of the stirrers in the kettle was sufficient for this purpose; as the pimientos were added when the temperature was between 52° and 57° F. and the cheese mass drawn from the kettle at 68° C., the agitation received was not excessive. All samples were made up with 2.0 per cent of sodium citrate and 0.5 per cent common salt.

The additions shown in the table were made in an effort to study the effect of small changes in the reaction and to meet the criticism that "1" type was a trifle acid, or sour to the taste.

After one week in storage the samples were examined for preference by various disinterested parties as regards taste and general appearance and at this time analyses were made for the moisture in the cheese, the titratable acidity calculated as lactic acid, and the hydrogen-ion concentration as determined by the hydrogen electrode. These results are shown in table 1.

From the figures obtained it is evident that there was very little difference in the pH of the samples, although the titratable acidity varied—samples No. 2, No. 3 and No. 4 being more acid than the others. That the difference in pH was of little importance as far as the examiners were concerned was shown by the fact that No. 6, which was the most acid in terms of pH, was one of those receiving preference, as were also No. 9 and No. 10.

In the examination of the samples the men were asked to indicate their preference in regard to (a) the color of the cheese mass and the pimientos, (b) the distribution and size of the pimiento pieces, and (c) the flavor. Figure 1 illustrates the difference in the samples with respect to the size of the pimiento pieces. From the general appearance nearly all of the judges chose sample No. 8, but its very peculiar taste eliminated it from further consideration. One of the examiners stated that it tasted to him as though it contained some old oil. This led to a review of the preparation of pimientos and it was found that they are prepared for canning by roasting, which is done either over an open fire or by dropping the raw peppers into a bath of hot oil. This latter procedure would no doubt explain the very peculiar and oily flavor found in sample No. 8. It was further learned that commercially the fire-roasted peppers are used and these can be identified by the presence of small black pieces of material due to the insertion of the roasting fork.

Sample No. 1 without pimiento was used only for purposes of comparison to show the increase or change in color due to the addition of the pimientos to the other samples. The general preference of the judges seemed to be either No. 7 or No. 9 with No. 5, No. 6 and No. 10 considered as very satisfactory. The off flavor of No. 8 eliminated it. Samples No. 2, No. 3



PIMIENTOS IN PROCESS CHEESE

Top row	Control.
Second row from top	samples No. 2, No. 3, No. 4.
Third row from top	samples No. 5, No. 6, No. 7.
Bottom row	samples No. 8, No. 9, No. 10.

and No. 4 were criticized as having a rather weak color both in the cheese mass and the pimientos. In sample No. 5 the color and size of the pimientos pieces were comparable with the succeeding samples.

There is not sufficient information available on the subject of pimientos to warrant any discussion as to why certain types break up very easily into small pieces that fail to have the desired eye appeal to the consumer.

SUMMARY

From the preference shown there is a decided eye appeal in processed cheese containing pimientos in which the pieces of pimientos are of a bright red color and of such size as to be readily seen.

The use of fire-roasted peppers will eliminate the possibility of off flavors that are due to oil roasting, either because of the use of old or rancid oil or the incomplete removal of the oil after roasting.

The authors wish to thank all of those who so kindly examined the samples submitted to them and offered their comments. This work was conducted under the fellowship sponsored by Chas. Pfizer and Company, Inc., to whom the authors also wish to express their appreciation.

THE INFLUENCE OF CITRIC ACID UPON TITRATABLE ACIDITY AND HYDROGEN-ION CONCENTRATION OF FROZEN DESSERTS

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The manufacture and sale of frozen desserts resembling ice cream, but which do not comply with legal standards for ice cream, prompted legislation defining various frozen desserts and has emphasized the need for exact methods of differentiating between these products. It is difficult to make certain that ice milk is not sold as ice cream, for the consumer may not judge the fat content by richness of flavor only and the inspector may not be certain whether a product was sold for ice milk or ice cream. In New York this problem has been handled by legislation forbidding the sale of ice milk.

The identity of ices is evident as they do not contain milk solids and are composed essentially of sweetened frozen fruit juices. On the other hand, sherbets contain milk solids as well as fruit juices and there is greater danger of confusing the identity of the products unless there is a detectable difference in acidity.

The "Suggested Ice Cream Law" of the International Association of Ice Cream Manufacturers¹ specifies that a "milk sherbet" and "ice or ice sherbet" shall contain not less than 0.4 per cent of titratable acidity expressed as lactic acid. The authors have favored a relatively low titratable acidity standard, for the most satisfactory flavor in ices and sherbets was found by Dahlberg² to have titratable acidities from 0.35 to 0.40 per cent expressed as lactic acid. It is recognized, however, that higher acidities are usually found in the commercial products, but the trend is toward lower acidity. Turnbow and Raffetto³ although recommending the addition of liberal quantities of fruit also specified the equivalent of 0.38 to 0.44 per cent of citric acid crystals. Sommer⁴ recommends the equivalent of 0.12 to 0.31 per cent of citric acid which agrees well with the 0.20 per cent suggested by Dahlberg for orange ice or sherbet. In 1930 Caulfield and Mar-

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¹ Suggested Ice Cream Law by Definition and Standard Committee of International Association of Ice Cream Manufacturers.

² A study of the manufacture of water ices and sherbets. N. Y. Agric. Exp. Sta. Bul. 536. 1926.

New developments in methods used in making ices and sherbets. Ice Cream Trade Jour. April, 1930.

³ Ice cream. John Wiley and Sons. 1928.

⁴ Ice cream making. Published by the author, Madison, Wisconsin. 1932.

tin⁵ suggested a formula for sherbet containing 0.50 per cent citric acid, based upon analysis of 28 commercial sherbets. This brief statement of recommendations clearly emphasizes the marked variations in practice and the need for caution in establishing standards.

The present investigation was planned to establish the relationship between titratable acidity and pH with the hope that a more satisfactory basis for differentiating sherbets from ice milk or ice cream would be found in the pH values.

CITRIC ACID

There is much confusion regarding the strength of citric acid as used in the dairy industry. Citric acid crystallizes from solution with one molecule of water and has a molecular weight of 210. The moisture content is 8.57 per cent. Since it is trivalent the combining weight of the hydrated crystals is 70. Upon exposure to the air the hydrated crystals lose their water of crystallization but always retain some moisture, although for practical purposes the water held when in equilibrium with average atmospheric conditions is of little significance. Completely dehydrated citric acid has a molecular weight of 192 and a combining weight of 64.

It is essential to know the moisture content of the citric acid or of the citric acid solution to be able to accurately titrate an ice or sherbet for total acidity and calculate the amount of citric acid required to raise the acidity to the desired degree. Citric acid is generally sold to the ice-cream trade as monohydrated granules or large crystals. The hydrated citric acid is not in equilibrium with ordinary atmospheric conditions and, when not in an air tight container, loses water.

One pound of hydrated granular citric acid was secured in a glass container stoppered with a cork. A sample of the crystals dried to constant weight in a vacuum oven at 45° C. for approximately 72 hours showed 8.45 per cent of water against a theoretical moisture content of 8.57. The bottle was left stoppered for 24 days when the hydrated crystals were used experimentally. The moisture content had decreased to 7.53 per cent. Twenty grams of these crystals were placed in an aluminum drying dish, protected against dust, and weighed weekly or more often. At the end of the experiment a moisture test was made on the citric acid.

The data in figure 1 show the very rapid loss of moisture from hydrated citric acid exposed to atmospheric conditions in small quantities. The air temperature varied from 20 to 25° C. and the humidity from 10 to 50 per cent. No data were secured to associate humidity with moisture loss. Within a month the citric acid contained only 0.1 per cent of water and it held constant at this moisture content. Undoubtedly this loss of moisture was more rapid than would be secured under commercial conditions for the

⁵ What goes into the manufacture of a high grade sherbet. Ice Cream Trade Jour. May, 1930.

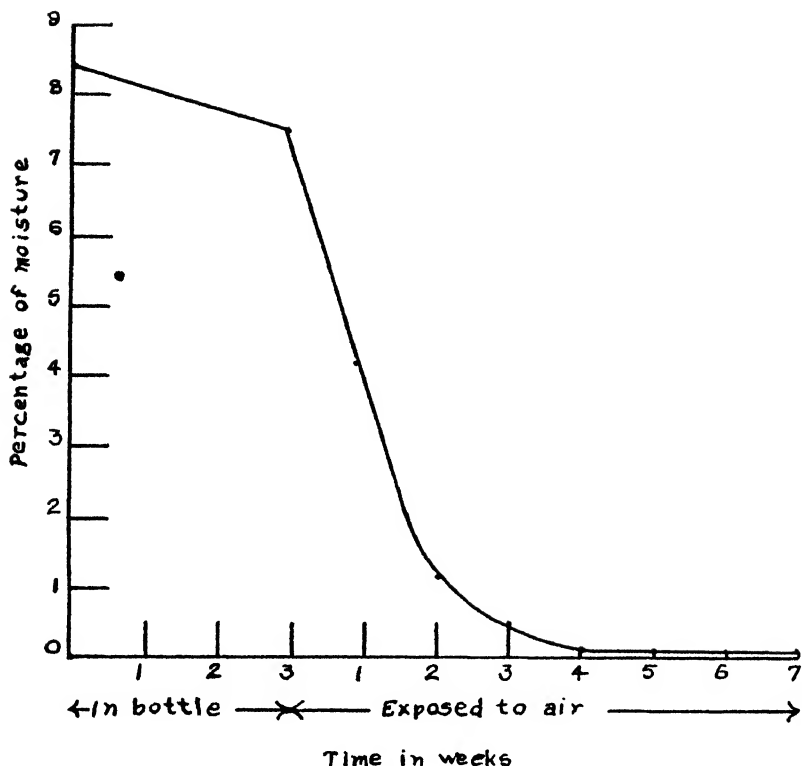


FIG. 1. MOISTURE LOSS FROM MONOHYDRATED CITRIC ACID CRYSTALS

volume of hydrated acid crystals would be larger, the crystals would be somewhat protected against free movement of air, and the humidity of the room might be higher. In correspondence, Chas. Pfizer and Co. have stated that more than 100 days would be required to dry hydrated granular or crystal citric acid to equilibrium at a moisture content of 0.4 per cent. The citric acid in these tests was in equilibrium at 0.1 per cent and no explanation for this discrepancy in the moisture content at equilibrium is evident.

Since dehydrated citric acid is now available at prices which compare with those for hydrated citric acid, the former product is to be preferred as it is almost constant in strength and its use is more convenient.

The amount of citric acid to be added to an ice or sherbet can be calculated from titration data with considerable accuracy. For example, if 9 grams of ice are titrated with N/10 sodium hydroxide the number of cubic centimeters of neutralizer used divided by 10 gives the percentage of lactic acid. If 6.4 grams are titrated the number of cubic centimeters divided by 10 gives the percentage of dehydrated citric acid. If 7.0 grams are titrated the number of cubic centimeters divided by 10 gives the percentage of hydrated citric acid. The percentage of lactic acid may be converted to

dehydrated citric acid by multiplying by 64/90, or to hydrated citric acid by multiplying by 70/90. Unless moisture tests are made regularly on the hydrated crystals it is essential to use the dehydrated product for uniform results.

In preparing the customary 50 per cent citric acid solution, it is necessary to consider the moisture content of the acid. For example, 4 pounds of dehydrated citric acid + 4 pounds of water will make a 50 per cent citric acid solution but 4.37 pounds of the hydrated crystals would need to be added to 3.63 pounds of water to give 8 pounds of a solution of equal strength.

INFLUENCE OF CITRIC ACID UPON ACIDITY

The ices used in these experiments were a 30 per cent solution of sugar (sucrose) in water. The sherbets contained 50 per cent of milk testing 4 per cent of fat, 30 per cent of sugar, and the balance was water. The ice milk contained 65 per cent of 4 per cent milk, 5 per cent of dry skim milk, 15 per cent of sugar and the balance was water. Its serum solids content compares favorably with that present in ice cream, so that data secured with ice milk would be interchangeable with that secured on ice cream. To each product 0.1, 0.2, 0.3, 0.4, and 0.5 per cent of dehydrated citric acid was added.

Nine grams of each solution were titrated with N/10 sodium hydroxide, using 5 drops of a one per cent alcoholic solution of phenolphthalein as indicator. The pH of the solutions was determined electrometrically, using the quinhydrone electrode and colorimetrically using the method of Sharp

TABLE 1

Relation of titratable acidity and hydrogen-ion concentration in ice milk, sherbets, and ices containing varying percentages of citric acid

CITRIC ACID	ICES			SHERBET				ICE MILK			
	Titratable acidity		pH	Titratable acidity		pH		Titratable acidity		pH	
	Lactic	Citric	Elect.	Lactic	Citric	Elect.	Color.	Lactic	Citric	Elect.	Color.
%	%	%		%	%			%	%		
0	0.02	0.01	7.25	0.07	0.05	6.32	6.2	0.20	0.14	6.21	6.1
0.1	0.13	0.09	3.11	0.21	0.15	5.60	5.4	0.34	0.24	5.76	5.7
0.2	0.28	0.20	2.74	0.34	0.24	4.90	4.8	0.48	0.34	5.44	5.4
0.3	0.42	0.30	2.57	0.47	0.33	4.53	4.6	0.62	0.44	5.10	5.0
0.4	0.58	0.41	2.45	0.61	0.43	4.13	4.5	0.77	0.55	4.84	4.8
0.5	0.72	0.51	2.39	0.73	0.52	4.00	4.5	0.90	0.64	4.67	4.7

and McInerney.⁶ It is evident that for certain colored fruit products some difficulty may be experienced with the latter method.

The results secured on two trials which are typical of other tests are summarized in table 1 and graphically represented in figures 2 and 3. In figure 2 the titrations are expressed in terms of dehydrated citric acid and

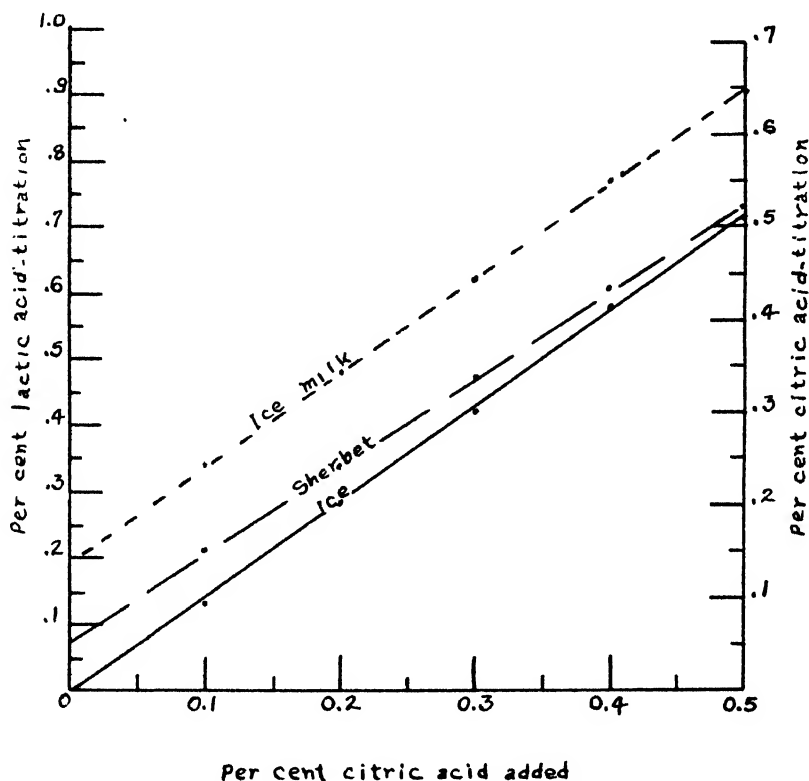


FIG. 2. RELATION OF TITRATABLE ACIDITY, EXPRESSED AS DEHYDRATED CITRIC ACID, TO DEHYDRATED CITRIC ACID ADDED TO FROZEN DESSERTS AND LACTIC ACID

lactic acid. The data are of special interest in showing that, contrary to several statements in the literature, the titration of a water-ice correctly shows the amount of dehydrated citric acid added. The titratable acidity of ices, sherbets, and ice milk increase in direct relation to the acid added. It is evident that results are relatively similar and that for the purpose of the ice-cream industry there is little choice between expressing the results as lactic or citric acid except that above the initial acidity it is citric acid that is being titrated and used to increase the acidity even though it is derived from fruit juice such as lemon or orange.

⁶ The colorimetric determination of the hydrogen-ion concentration of milk, whey, and cream. Jour. Biol. Chem. 70: 729-758. 1926.

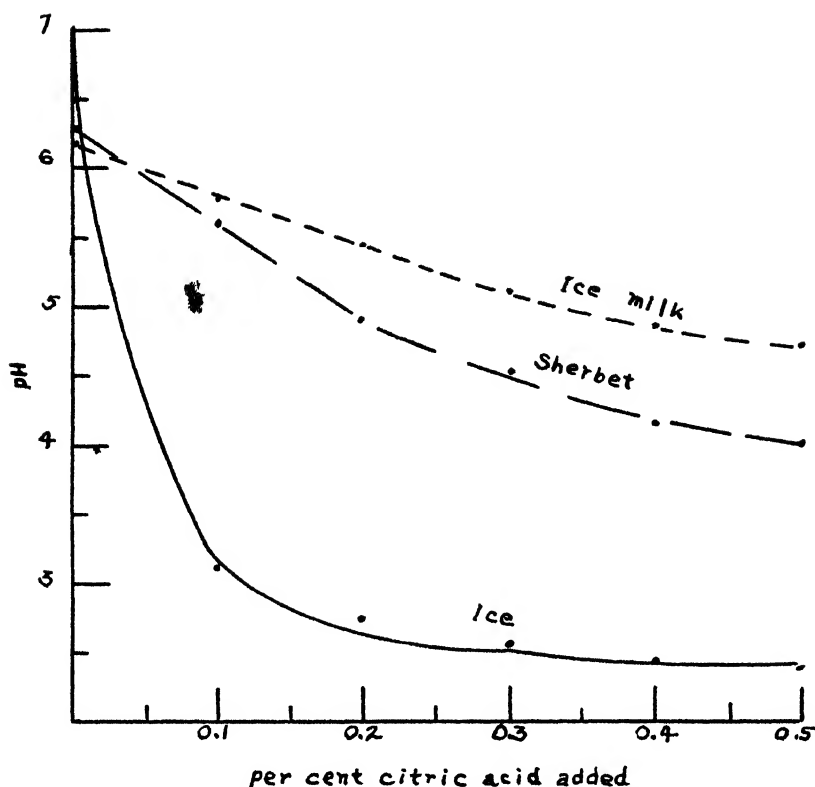


FIG. 3. RELATION OF HYDROGEN-ION CONCENTRATION TO DEHYDRATED CITRIC ACID ADDED TO FROZEN DESSERTS

The curves in figure 3 show the pH of these various products and represent distinct differences in the decrease in pH due to added acid. This difference is due to the buffer action of certain of the milk solids in retarding the increase in hydrogen ions as citric acid is added. These data have a special value in differentiating sherbets from ice milk or ice cream which will be discussed later.

DISCUSSION OF DATA

These studies have clearly demonstrated the need of exact information regarding the moisture content of citric acid in crystalline or powdered form. The monohydrated crystals or granules contain 8.5 per cent of moisture which is lost at a more or less rapid rate, thereby increasing in strength. Anhydrous or dehydrated citric acid contains about 0.1 per cent moisture and is rather constant in strength. Its use is generally to be preferred for this reason.

The addition of citric acid to ices, sherbets and ice milk affects the pH and titratable acidity in accordance with expectations and there is every

possibility of accurately and uniformly standardizing the acidity or pH of these products.

The problem of differentiating ices and sherbets from ice milk and ice cream on the basis of acidity is entirely feasible. Such distinction cannot be made satisfactorily on the basis of total titratable acidity. There appears to be marked variations in consumer preference for different acidities which may be affected by the sugar content of the sherbets and ices, by the season of the year, and by the locality. Former data from these laboratories were verified in that 7 out of 8 persons preferred sherbets and ices with a titratable acidity, expressed as lactic, of less than 0.4 per cent. This corresponds to the addition of approximately 0.2 to 0.25 per cent of dehy-

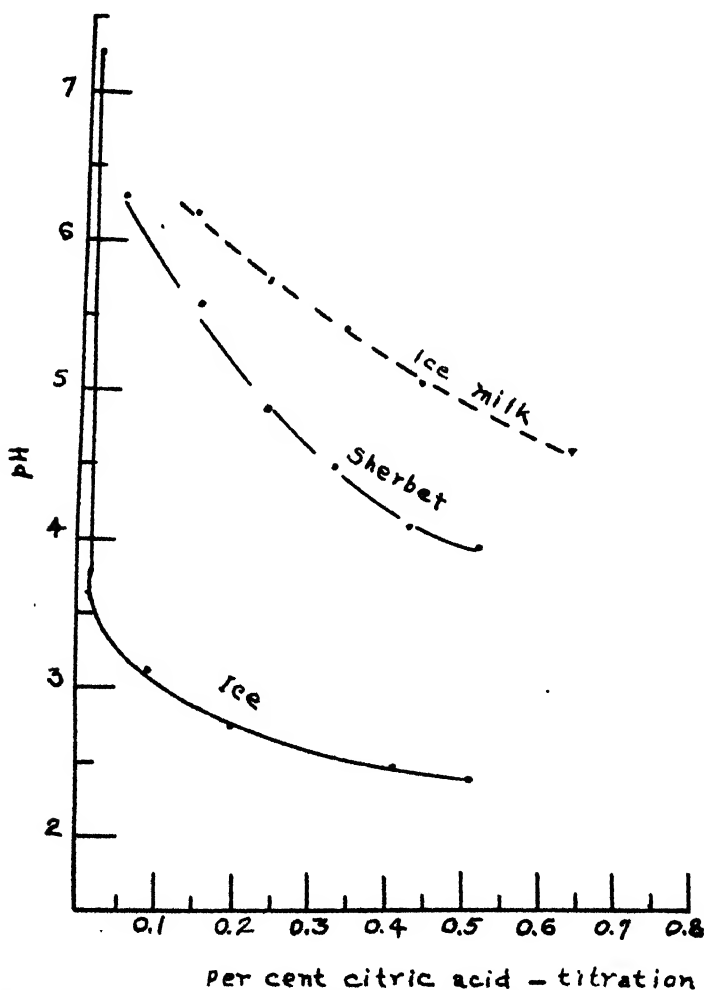


FIG. 4. RELATION OF HYDROGEN-ION CONCENTRATION OF FROZEN DESSERTS TO TITRATABLE ACIDITY, EXPRESSED AS DEHYDRATED CITRIC ACID

drated crystals. To permit the manufacture of these products with a desired acidity the titratable acidity, expressed as lactic, for ices and sherbets would need to be set at a minimum of 0.35. Ice milk or ice cream with high percentages of serum solids would approach this standard and there might be difficulty in differentiating the sweet frozen desserts from the frozen desserts which were sour due to fruit juices or acids but which were low in serum solids.

It is possible, however, to clearly distinguish ice milk and ice cream from sherbets and ices on the basis of pH. In figure 4 the relationship between titratable acidity, expressed as dehydrated citric, and pH is shown. If a standard of pH 5 or less is adopted for ices and sherbets the figure shows that ice cream would need to have a titratable acidity of more than 0.50 per cent, the equivalent of 0.7 per cent lactic acid, to be considered as a sherbet or ice. Such ice cream would be curdled, sour, and unsalable. On the other hand, an ice could titrate only 0.1 per cent citric acid and a sherbet could titrate only 0.25 per cent and still have a pH value of less than 5. Expressed in another manner, it can be seen from figure 3 that to comply with this standard the citric acid content of an ice would need to be only 0.1 per cent, of a sherbet only 0.2 per cent but 0.4 per cent citric acid would need to be added to ice cream to have a sufficient low pH to pass as a sherbet. It is clearly evident that sweet ice cream cannot be successfully made with a pH of 5 but relatively sweet ices and sherbets can be made with a pH of 5 or less. Such a standard could be used for differentiating these products and would be especially helpful in determining whether a sample of the chemical composition of a sherbet was sold for ice milk, and *vice versa*.

CONCLUSIONS

1. The monohydrated citric acid crystals, granules, or powder lose moisture more or less rapidly under atmospheric conditions. The original moisture content of 8.5 per cent. decreases to 0.1 per cent. As this is the moisture content of dehydrated citric acid, more consistently uniform results can be secured by using the acid in the dry form.

2. The citric acid added to water ices can be accurately accounted for by direct titration but in the case of sherbets and ice cream the results are consistent only when allowance is made for the alkali required to neutralize the dairy products.

3. The pH of frozen desserts is affected by added citric acid depending upon the buffer action of the milk solids. Since sherbets and ices are acid fruit products with no or low percentages of milk solids, their pH is below 5 while that of ice cream and ice milk is above 6. A standard of pH 5 or below for sherbets and ices is reasonable and could be used to distinguish them from sweet frozen desserts like ice cream or ice milk.

CHEESE SPREADS. II*

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In a previous paper¹ the subject of cheese spreads was discussed in a general manner with special emphasis on the fat and moisture content in relation to the spreading quality. In this paper a study will be presented of the effect of added milk solids in the form of either skimmilk or whey powder, a comparison of the emulsifiers commonly used and the effect of the age of the cheese on the texture and body of the finished product. Analyses of the samples will be presented to show the range of composition that was studied. A few analyses of commercial samples and of foreign cheese spreads will be included to show the range of composition of cheese spreads now available. The various samples were examined by men in the industry and men interested from the standpoint of control.

In addition to cheese spreads, the terms cheese foods or cheese food compounds are coming into use to designate products of this type. Some manufacturers prefer to use the latter terms because they are more descriptive of the product. Informative labels and advertising usually stress the addition of milk solids.

At the present time there are two rather distinct types of cheese spreads of domestic production available in the average retail store. The first is the type described previously in which the base is cheese with milk solids added either as skimmilk powder or whey powder and with sufficient water to give the desired consistency. In the other type the base is cream or cream cheese with enough well aged cheese added to give the finished product the desired flavor; in order to increase the body, milk solids may be added in dry form. Both types of cheese spread contain approximately 2 per cent of the emulsifying salt,—sodium citrate, Rochelle salt, or di-sodium phosphate, either separately or in combination.

In the previous paper¹ the attempt was made to keep the composition of the samples described approximately the same as that of process cheese. The spreads described in this paper have a wider range of composition and were more nearly of the same general composition of those of type 1 found on the market. In addition there are some samples that represent extremes in composition especially in regard to the amount of milk solids added.

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¹ TEMPLETON, HUGH L., and SOMMER, H. H. Cheese spreads. JOURNAL OF DAIRY SCIENCE, Volume 15, page 155, 1932.

Table No. 1 gives the composition of the cheese spreads that were made in this study. Samples No. 17 and 18 were made of cheese approximately 2 years old; samples No. 19 and 20, cheese 4 months old; all other samples an equal mixture of the two. The fat content was adjusted to the desired point by the addition of freshly churned, sweet cream butter. The samples were prepared with varying contents of skimmilk powder and whey powder so that in a comparison of the two there were comparable lactose contents. On the basis of the preference indicated by the judges, seven of the samples were analyzed for lactose, and the results are given in table No. 2.

In the absence of a score card or other recognized standards for products of this type, judges were requested to make their criticisms primarily on the body, texture, and spreading quality of the different samples without regard to the flavor unless it was so pronounced that they felt that the product would be discriminated against by the average consumer. The samples were examined when they were about one week old, again at two months, and finally after six months' storage. The number of men participating at each examination varied from 5 to 8, and none of them had any knowledge of the exact make-up of the samples that they were considering. At the two months' examination, the cheese food compounds were allowed to remain at room temperature without the tinfoil wrapper for twenty hours after the first examination and then a second inspection was made by one of the authors in order to note which of the samples had held their shape and which ones showed leakage of fat.

From table No. 1 it is possible to obtain a fair picture of the composition of each of the samples and the general reaction of the examiners. Samples 17 and 18 were made up of all old cheese while numbers 19 and 20 contained only the young cheese; none of these samples met with approval, the older cheese being too old, firm, mealy and failing to spread satisfactorily while the young cheese was lacking in flavor and its very rubbery texture made it very difficult to spread. The samples that were made up with sodium citrate were more satisfactory than the others because there was no fat leakage except in the very old cheese and all the samples held their shape very well except those containing large amounts of whey powder, in these samples it was quite evident from the increasing softness with the increasing whey powder that the emulsifying agent was not the responsible factor. All of the samples preferred by the examiners contained 2 per cent of sodium citrate. The analyses of these samples are shown in table 2.

With Rochelle salt the spreads were not as satisfactory as those containing citrate; while most of them had a good body, the rubbery texture made spreading difficult. The fat leakage on standing with No. 28 was expected on the basis of the examination of the other samples; this defect was not noted in any of the other samples in the Rochelle salt series. As was expected from the studies on processed cheese in which di-sodium phosphate

TABLE 1
Experimental cheese spreads

SAMPLE NO.	COMPOSITION OF CHEESE SPREADS					SUMMARY OF JUDGES' COMMENTS			
	WATER %	FAT %	FAT IN WFS %		MILK SOLID %	EMULSIFIER		FLAVOR	BODY AND TEXTURE
			%	Kind		%	Kind		
1	40.55	30.65	51.55	3.0 W	2.0 C	O. K.	Firm	O. K.	Fair
2	38.50	30.10	48.94	6.0 W	2.0 C	Trifle bitter	Firm	O. K.	Fair
3	40.40	30.53	41.22	10.0 W	2.0 C	Rather sweet	Weak	Trifle coarse	Good
4	41.80	25.50	43.81	10.0 W	2.0 C	Sweet	Weak	Trifle coarse	Good
5	40.40	29.42	49.36	6.0 W	2.0 C	Sweet	Trifle weak	Trifle coarse	O. K.
6	36.76	30.79	48.69	5.0 W	2.0 C	O. K.	Firm	O. K.	Poor
7	39.50	30.08	49.68	15.0 W	2.0 C	Too sweet	Soft	Coarse	Poor
8	44.93	26.50	48.12	12.0 W	2.0 C	Too sweet	Soft-weak	Poor	Too soft, lost shape
9	41.43	27.11	46.29	3.8 S	2.0 C	O. K.	Firm	O. K.	Lost shape
10	40.50	28.40	47.74	7.6 S	2.0 C	Trifle flat	Firm	O. K.	Held shape
11	40.39	28.65	48.06	13.0 S	2.0 C	Trifle sweet	O. K.	O. K.	O. K.
12	39.88	30.35	50.48	20.0 S	2.0 C	Sweet	O. K.	Smooth	Good
13	39.86	28.88	48.02	4.6 S	2.0 C	O. K.	O. K.	Fair	O. K.
14	41.29	25.99	44.34	10.0 S	2.0 C	O. K.	O. K.	Smooth	A trifle firm
15	40.92	25.41	43.01	9.2 S	2.0 C	Lacking	Firm	O. K.	Rather firm
16	44.20	22.70	40.68	12.0 S	2.0 C	Trifle sweet	O. K.	O. K.	Too firm—rubbery
17	40.44	30.28	50.84	6.0 W	2.0 C	Bitter, sharp	Firm	Poor	Good
18	38.27	30.64	49.64	8.0 S	2.0 C	Strong tainted	Firm	Poor	Too firm—greasy
19	39.33	27.58	45.46	5.9 S	2.0 C	Lacking, curdy	Firm	Rubbery	Too firm—greasy
20	43.50	28.33	50.14	7.3 S	2.0 C	Inspid, lacking	Firm	Rubbery	Poor
21	37.31	31.79	50.71	6.0 W	1.0 C	Off flavor	O. K.	Coarse	Lost shape
22	40.73	27.85	47.16	6.0 S	1.0 C	Poor flavor	Firm	Rubbery	O. K.
23	37.67	29.19	46.83	6.0 W	3.0 C	Fair	O. K.	Fair	Too firm
24	41.76	28.26	48.52	6.0 W	1.0 P	Bitter, sharp	Weak	Grainy	Fat on top, poor appearance
25	39.67	31.58	52.35	6.0 W	2.0 P	Bitter, sharp	Weak, crumbly	Grainy	Coarst, fat leakage
26	38.20	30.03	48.59	6.0 S	2.0 P	Bitter, sharp	Firm	Grainy	Fat leakage
27	39.59	30.23	50.04	6.0 W	3.0 P	Bitter	Weak	Sticky	Slight fat leakage
28	39.21	28.64	47.11	6.0 W	1.0 R	Off flavor, poor	Weak	Mealy	Poor
29	38.39	28.73	46.62	6.0 W	2.0 R	Trifle sharp	O. K.	Smooth	Coarst, fat leakage
30	38.38	28.46	47.18	6.0 S	2.0 R	Trifle sharp	O. K.	Rubbery	Trifle rubbery
31	38.80	29.00	47.39	6.0 W	3.0 R	Sharp	O. K.	O. K.	Trifle rubbery

The per cent of whey powder or skimmilk powder used is based on the weight of the powder as compared to the total weight of the ingredients used.
W—whey powder; S—skimmilk powder; C—sodium citrate; P—di-sodium phosphate; R—Rochelle Salt.

TABLE 2
Analyses of Cheese Spreads

SAMPLE NO.	WATER %	FAT %	FAT IN WFS %	LACTOSE		
				%	% IN WFS	PER 100 PARTS OF WATER
4	41.80	25.50	43.81	8.13	13.97	19.45
5	40.40	29.42	49.36	4.83	6.10	11.96
10	40.50	28.40	47.74	4.04	6.79	9.98
12	39.88	30.35	50.48	10.91	18.15	27.37
13	39.86	28.88	48.02	3.12	5.19	7.83
14	41.29	25.99	44.34	5.43	9.10	13.15
16	44.20	22.70	40.68	6.68	11.97	15.11

TABLE 3
Analyses of Commercial Spreads

SAMPLE (BRAND)	WATER %	FAT %	FAT IN WFS %	
A	42.00	26.67	45.99	
A*	42.93	26.33	46.13	
B*	43.87	25.05	44.62	
C*	45.18	23.59	43.03	
C	42.86	25.25	44.19	
D*	49.97	30.71	61.38	
E*	53.42	16.00	34.35	A foreign made Swiss spread made from partly skimmed milk
F*	65.28	7.52	21.66	A foreign made Swiss spread made from partly skimmed milk
G*	42.09	27.00	46.62	Foreign type process Swiss cheese
H*	41.89	32.99	56.77	Foreign type Swiss cream cheese
I*	44.35	31.87	57.27	Foreign Camembert cheese
J*	54.83	17.16	37.99	Foreign type cheese spread sold in metal tube

* These data furnished by the courtesy of Mr. Harry Klueter, Wisconsin Department of Agriculture and Markets.

was used, the cheese food compounds with this salt as emulsifier were rather weak in body and showed considerable fat leakage. Sample No. 24 with only 1.0 per cent of emulsifier had a layer of fat on the upper surface that gave the spread a very unattractive appearance. With the larger amounts of the emulsifier there was no free fat on top, but there was a decided loss of fat from the cheese spread when it was left at room temperature for any length of time.

In the comparison of the two forms of added milk solids, it was interesting to note from table 2 that five of the seven samples receiving preference were made with skimmilk powder. The cheese food compounds containing whey powder were noticeably weaker in body and it was very inter-

esting to note the difference in the rate at which they lost shape when the supporting tinfoil was removed. With samples No. 7 and No. 8, as soon as the tinfoil was removed, the cheese spread began to flatten out until at the end of four or five hours they had lost all semblance of their original rectangular shape. The samples with whey powder had a characteristic sweet taste that became rather too pronounced and somewhat disagreeable when more than ten per cent of whey powder was used. With the higher concentrations of either of the dry powders some of the examiners noted that the samples were a trifle gritty indicating the formation of lactose crystals, but this comment was not unanimous and a second careful examination of the cheese spread failed to show any microscopic crystals.

In view of the amount of lactose that was present in some of the samples and in view of our previous result¹ it was expected that lactose crystals would be found. This led to a careful review of the methods. In the earlier work the cheese mass was not drawn from the kettle as soon as the desired temperature was reached, but the stirring was continued for some time as some more cheese had to be added to give the mass in the kettle the desired consistency. In all the samples described in this paper the cheese mass was drawn from the kettle at 150° F. without further agitation and this no doubt accounts for the failure to have large crystals form. In discussing this question with a commercial processor, it was found that this conclusion is in harmony with practical experience.

Table No. 3 presents a few analyses of commercial samples of cheese food compounds. At the present time there are a number of foreign types of process cheese and cheese spreads on the market and this table is included to indicate their composition. Brand "D" represents type No. 2, a cheese food compound made up on a cream cheese base. With cheese spreads of the foreign types it is often stated on the label that they "spread like butter."

Cheese food compounds seem to meet a demand on the part of the consuming public. It is reported that the cheese used in the manufacture of these products amounts to more than twenty million pounds per year. It would seem that any product using such an amount of cheese and other dairy products is worthy of study and encouragement.

SUMMARY

Whey powder tends to give a weaker body to cheese spreads than skim-milk powder, but it also gives a rather distinctive sweet taste that may be very agreeable to some consumers.

When used in the proper proportions skimmilk powder gives a product that seems to meet with quite general approval. The cheese food compound can be spread easily, or, if occasion demands, may be sliced in a satisfactory manner.

The use of sodium citrate gave a very satisfactory product as there was no fat leakage except with very old cheese and the body of the samples containing this salt was more uniform than that produced with either Rochelle salt or di-sodium phosphate. With the latter salt there was a greater tendency to fat leakage than with either of the other emulsifiers.

In order to obtain a satisfactory product it is advisable to blend cheese using some old cheese for the flavor. The use of very old cheese alone is apt to give a product that is grainy and shows fat leakage, while with young cheese alone the finished product is too rubbery to spread easily.

The choice made by the different examiners indicates that preference varies with the individual taste. It is therefore impossible to give any composition formula that would meet with universal approval.

In conclusion the authors wish to thank all those men who examined the samples of cheese food compounds and offered their comments. This work was carried on under a fellowship sponsored by Chas. Pfizer and Company, Inc., to whom the authors also express their appreciation.

THE FAT PERCENTAGE OF MILK AS AFFECTED BY FEEDING FATS TO DAIRY COWS

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INTRODUCTION

The part played by the fat of the food in the formation of milk fat has been, for many years, a question of great interest to those concerned with problems of milk production. A large number of publications are found which have a bearing upon this question, some of them having appeared more than fifty years ago. A study of these papers gives no entirely satisfactory answer to the question, since the conclusions reached by the different investigators are not at all in agreement, although a number of experiments have given rather convincing evidence that the addition of certain fats to the ration will cause an increase in the fat content of the milk. The literature upon this subject is so extensive that no attempt is made to review it in this paper.

Jordan and associates (3) (4), feeding rations from which most of the fat had been extracted, showed very conclusively that nutrients other than fat may be the source of milk fat. Maynard and McCay (5), following a procedure similar to that of Jordan and associates, have recently found that replacing nearly all of the fat of the ration with an isodynamic amount of starch resulted in a marked decrease in the yield of both milk and butterfat. The fat percentage was found to be variable and no evidence was obtained to show that it was lowered by the reduced fat intake. There is no justification, however, for assuming that the same principles will hold true when the fat content of the ration is increased above that normally encountered.

The one case involves a deficiency for which the cow has a reserve in the form of body fat from which she may draw. Eckles and Palmer (2) found that when cows were forced to draw upon their reserve because of underfeeding, the fat content of the milk was increased. The condition produced by a ration deficient in fat might be somewhat analogous to that produced by underfeeding and it is conceivable that the fat content of the milk might even be increased by reducing the fat intake to extremely low levels.

In the case of addition of fat to a ration already adequate in fat content, a situation is created where there may be available to the mammary gland a relatively large amount of material which may be particularly well suited to the production of milk fat.

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Most of the studies upon the relationship of the fat intake to the fat content of the milk have been carried out by feeding substances of high fat content or by direct addition of fats or oils to the ration. It has been definitely shown that certain fish oils, when fed to dairy cows, will depress the fat content of the milk. The fish oils have characteristics markedly different from those of fats normally found in feedstuffs and constitute a problem in themselves. The conflicting results and conclusions of the experiments involving other oils and fats do not give a very satisfactory answer to the question, and for this reason it was considered desirable to undertake a more comprehensive study of this problem.

This paper deals primarily only with one phase of the problem, namely, the immediate effect of increasing the fat content of the ration. This immediate effect, in addition to its fundamental importance, has a very significant practical application in connection with the problems of advanced registry and dairy herd improvement association testing, where the yearly production of dairy cows is estimated from the production during test periods of one or two days' duration at monthly or bimonthly intervals. The accuracy of such estimates depends entirely upon whether or not the short test periods are truly representative of the average for the longer periods which they govern.

EXPERIMENTAL

In each of these experiments, the cows were divided into two groups as nearly alike as possible from the standpoint of breed, production, size, and condition. The periods were of six days' duration. A period in which the basal ration was fed always intervened between periods in which the fat was added to the ration. The two groups were alternated so that one was always receiving the basal ration while the other received the experimental ration. The ration was changed in each case with the evening milking of the last day of each period. The full effect of the change of ration usually did not appear until the second day after the change, although some effect often was noted on the first day. For this reason, the first day of each period was considered as a transitional period and the production during the remaining five days was used in calculating the average daily production.

The milk was tested for butterfat by the Babcock method. The samples were prepared by taking aliquot portions of the morning and the evening milking of each cow and mixing these two samples together, thus securing individual samples which were used as a basis for computing the individual daily butterfat production. The total milk and butterfat production of each cow was calculated for each five day period and from these figures the average butterfat percentage for the period was derived.

In assembling these data for presentation in graphical form, the total

milk production of Group 1 during the period of fat feeding was added to the total milk production of Group 2 during the following period when Group 2 received the same treatment. The milk production of the two groups during the corresponding control periods was likewise added. From these figures the average daily milk production was calculated for the experimental ration and likewise for the basal ration. The average daily butterfat production and the mean butterfat percentage were calculated in a similar manner. By this method of grouping, such influences as advancing stage of lactation, or effects of atmospheric conditions are largely compensated. In figures 1, 2, 3, and 4, the solid line connects the points representing the average performance of the two groups thus calculated during the portion of the experiment when they received the experimental ration. The broken line connects the points representing the average performance of the same animals during the portion of the experiment when they received the basal ration. This provides a basis for comparison regardless of any factors such as weather conditions or advance of lactation which might exert a specific influence upon both groups of animals.

The cows were at all times fed slightly above their requirements as calculated by Haecker's feeding standards, to eliminate the possibility of encountering the factor of underfeeding. Any necessary adjustments of the ration were made on the basis of the production during the control periods.

The fat was fed in every case by melting and pouring it over the grain mixture while it was still warm. It was then thoroughly mixed by rubbing between the hands until each particle of feed was coated with a film of fat and had an oily appearance.

No serious difficulty was encountered at any time in getting the cows to eat the feed to which the fat was added, nor were any digestive disturbances noted due to its presence.

Experiment I

It has previously been found (1) that the addition of large amounts of whole milk or cream to the ration of dairy cows caused a very marked increase in the butterfat content of the milk as the result of a corresponding increase in butterfat production. The fact that feeding skimmilk did not influence the fat content of the milk indicated that the fat of the whole milk or cream was responsible for this effect.

Theoretically, butterfat should be the ideal fat for causing increases in the fat content of milk since it supplies in the correct proportions all of the materials necessary for such an increase. When fed in the form of milk or cream it is in a finely emulsified form in which condition it might be more effective than if fed as an undispersed fat. Experiment I was planned to determine whether or not the butterfat has a similar effect if fed in an unemulsified form and also to find if other common fats have an effect similar to that of butterfat.

For this experiment the cows were fed alfalfa hay at the rate of 1 pound, and corn silage at the rate of 3 pounds, per 100 pounds of body weight. A grain mixture of 5 parts each of oats, barley, and corn; 2 parts wheat bran; and 1 part each of corn gluten meal, linseed meal, and cottonseed meal was fed according to the production of the animal. During the periods of fat feeding, the fat was merely added to the ration with no attempt to compensate for the additional energy intake. The fat was added to the grain mixture at the rate of 1 pound for each 10 pounds of grain. Thus, a cow receiving 10 pounds of grain during the control period received 10 pounds of grain plus 1 pound of fat during the experimental period. The additional fat thus received by each cow was approximately equal to her butterfat production. The experimental animals included two Holstein, two Jersey, and two Guernsey cows.

Linseed oil, lard, corn oil, cottonseed oil, tallow, and butterfat were fed in the order indicated. The linseed oil was a good grade of raw oil. The lard, corn oil, and cottonseed oil were refined products prepared for cooking purposes. The tallow was an edible grade of rendered beef fat. The butterfat was rendered from a good grade of fresh butter.

In order to determine whether or not the increased energy of the added fat was a factor, cane sugar was fed instead of fat to each group during one period. The sugar was added to the ration at the rate of 2.25 pounds to each 10 pounds of concentrates, thus securing an increase in energy intake comparable to that secured when the fat was added, without any change in the fat intake.

Figure 1 shows the behavior of the six cows included in the experiment. The individual cows responded in the manner characteristic of the group in every case except one. One Holstein cow, when fed 1.25 pounds of linseed oil, produced less butterfat and her milk contained a lower percentage of butterfat than during the previous or following period when she received only the basal ration. Every other cow in the group responded to the linseed oil with an increased content of fat.

No change in milk production which could be considered significant was observed. The butterfat production and percentage were decidedly increased during the periods of fat feeding, the full effect appearing usually on the second day after the fat was first fed. The cows promptly returned to their normal test when the fat was removed, the effect having practically disappeared by the second day after the fat feeding was discontinued.

The effect of all of the fats included in this experiment was quite similar with no significant indication that any one was more effective than the others. The experiment demonstrated conclusively that it is not necessary that the fat be fed in an emulsified form in order to secure the effect.

Increasing the energy intake by addition of carbohydrates in the form of cane sugar to a ration already adequate for the need of the cow had no

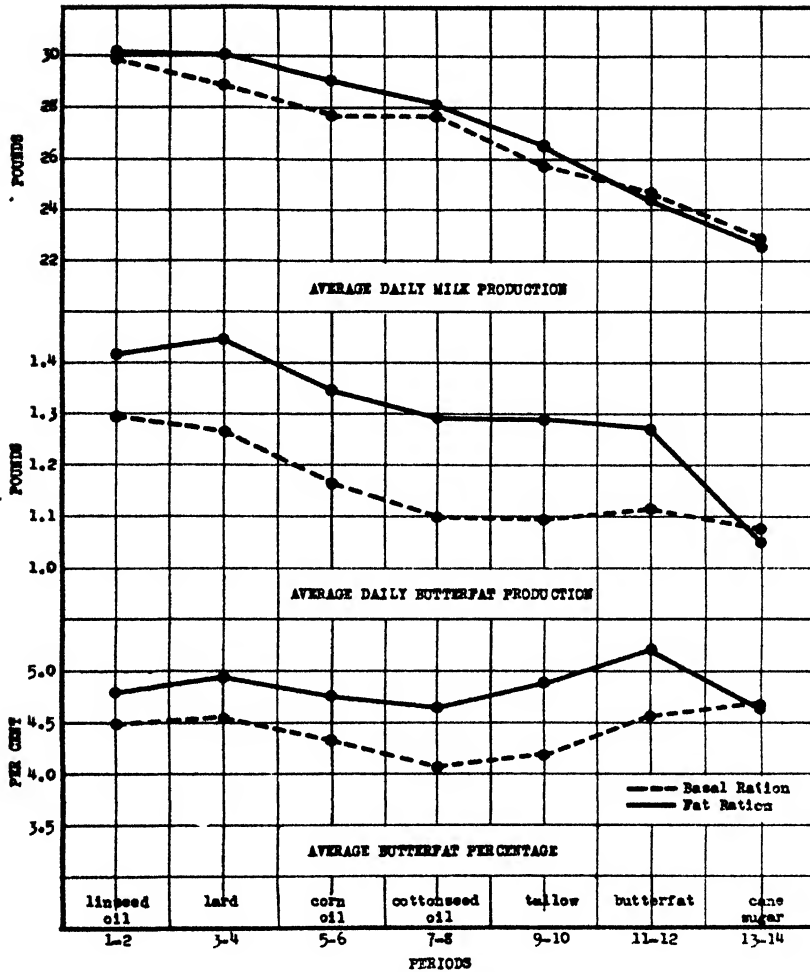


FIG. 1. MILK AND BUTTERFAT PRODUCTION AS INFLUENCED BY ADDITION OF VARIOUS FATS OR CANE SUGAR TO THE RATION. (Experiment I.) Average for two Holstein, two Guernsey, two Jersey cows by five day periods. Fats added during experimental portion of periods 1 to 12 at the rate of 1.0 pound per 10 pounds of grain. Sugar added during periods 13 to 14 at the rate of 2.25 pounds per 10 pounds of grain.

immediate effect upon milk or butterfat production nor upon butterfat percentage. This indicates that the effect of the food fats was not due to the additional energy which they supplied, but to the nature or properties of the fats.

Experiment II

This experiment was planned to study the relationship between the extent to which the fat intake is increased and the degree to which the fat content of the milk is affected. In this experiment and those following, the

energy intake was kept constant by decreasing the carbohydrates of the ration sufficiently to compensate for the fat which was added.

The cows were fed alfalfa hay as roughage. The grain mixture consisted of 2 parts barley, 1 part bran, and 1 part oats. In addition, 2 pounds of dried beet pulp were soaked and fed daily to each cow. Sufficient starch was fed during the control periods to equal, in energy value, the largest amount of fat which was to be fed. When fat was added, the starch was reduced sufficiently to keep the energy intake constant.

In determining the amount of fat to be fed to the individual cows, both the size of the cow and the level of production are factors which must be considered. Production being equal, the larger cow has a greater volume of blood, probably with a relatively small proportion circulating through the mammary gland where the circulation is no doubt related, to some extent, to the secretory activity. Consequently, by feeding a given amount of fat, the butterfat production of the larger cow might not be influenced to the same extent as the butterfat production of a smaller cow producing the same amount. Likewise, the cows being of the same size, the mammary gland of the higher producer would probably have available a greater proportion of the additional alimentary fat, since the more active gland would probably receive a relatively greater portion of the blood flow than would the gland in a less active state. This complicating factor was eliminated by selecting cows as nearly as possible of the same body weight and of the same butterfat production. Two Jersey and two Guernsey cows, averaging about 1000 pounds in body weight and varying by less than 75 pounds, were selected. Each was producing approximately 1.25 pounds of butterfat daily at the beginning of the experiment. Butterfat was fed at successive levels of 1.25 pounds, 0.25 pound, 0.50 pound, 0.75 pound, and 1.0 pound daily, with intervening periods on the basal ration. The cows were divided into two groups and the groups were alternated as before. The highest level of fat increase in the ration was equivalent to the butterfat production of the cows, or about 0.125 pound of fat per 100 pounds live weight. The lowest level was slightly more than the increase in butterfat production due to feeding the larger amount. This smallest amount was sufficient, if it were all recovered in the milk fat, to produce an effect as great as that secured by feeding 1.25 pounds daily.

Figure 2 shows the relative effects of the different levels of fat intake. There was no significant difference in the milk production. The butterfat percentage was definitely increased by raising the fat content of the feed. As the amount of fat in the ration was increased, there was an increased effect upon the fat content of the milk until, with the daily feeding of 1.0 pound of butterfat, the effect appeared to be fully as great as when the highest level of 1.25 pounds was given. The effect at the lowest level was slight but there could be little doubt that the difference was actually due to

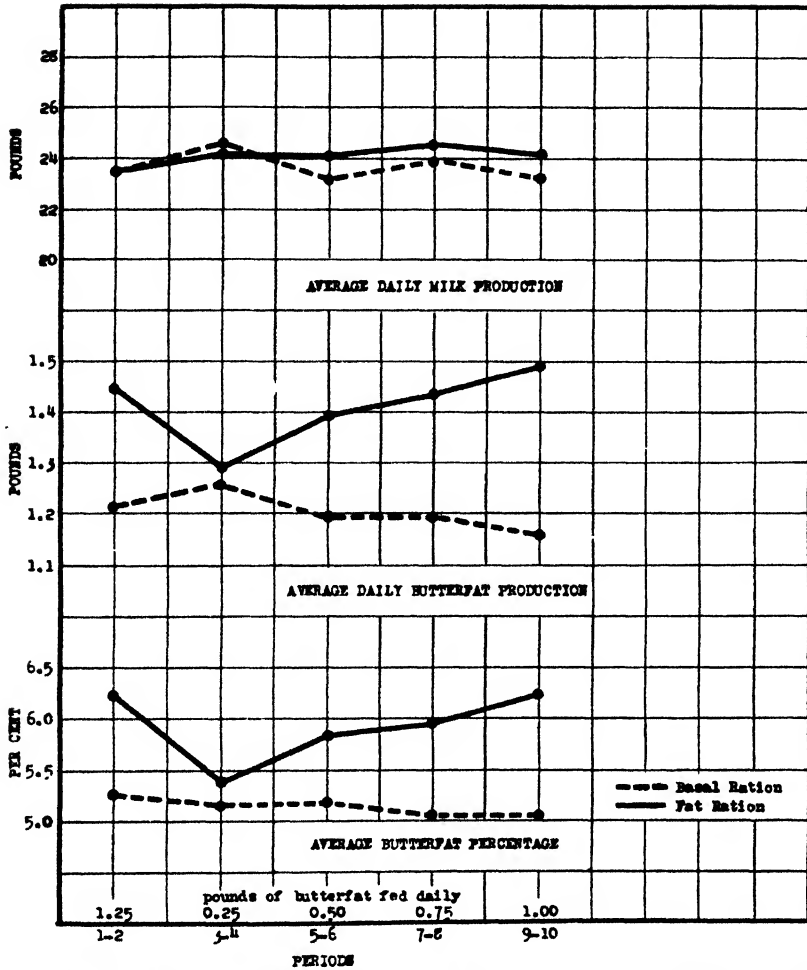


FIG. 2. MILK AND BUTTERFAT PRODUCTION AS INFLUENCED BY FEEDING DIFFERENT AMOUNTS OF BUTTERFAT. (Experiment II.) Average for two Guernsey and two Jersey cows by five day periods. Butterfat replacing starch in ration. Energy intake kept constant.

the difference in the fat content of the rations. Every cow responded in the manner characteristic of the group.

Experiment III

This experiment was conducted in the same manner as Experiment II with a similar group of two Jersey and two Guernsey cows and using the same ration except that raw linseed oil instead of butterfat was included at successive levels of 0.25, 0.50, 0.75, 1.00 and 1.25 pounds daily to each cow. Figure 3 shows the results of this trial. The behavior of every cow agreed with that of the group at the three higher levels. One Jersey cow when

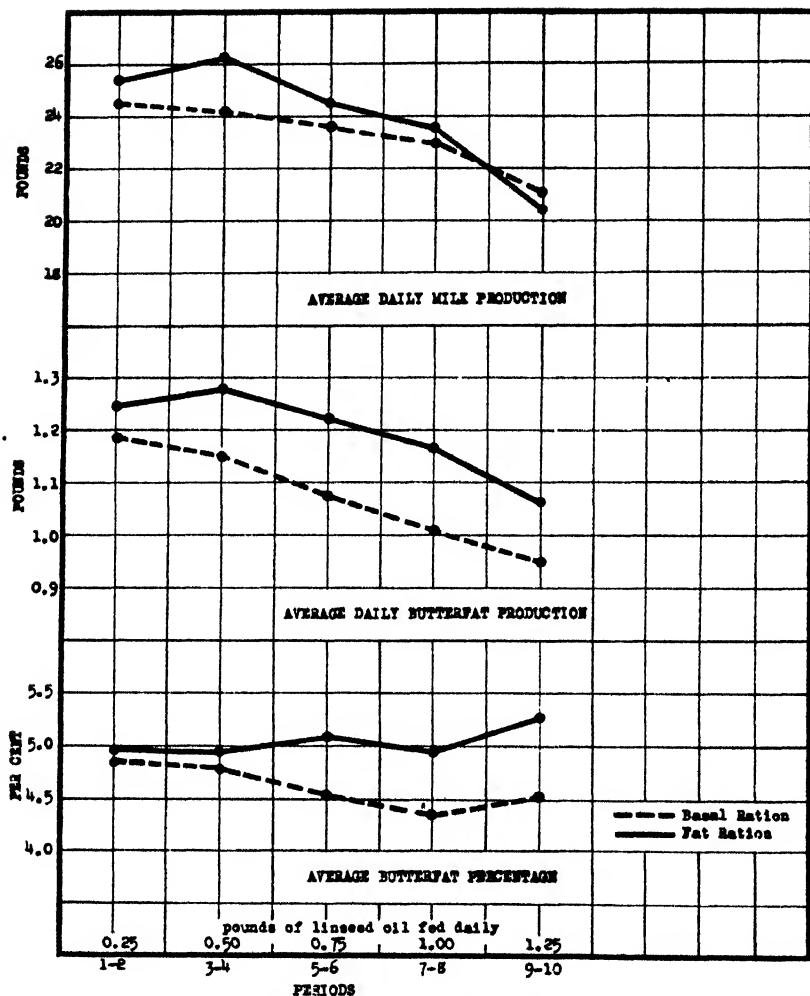


FIG. 3. MILK AND BUTTERFAT PRODUCTION AS INFLUENCED BY FEEDING DIFFERENT AMOUNTS OF LINSEED OIL. (Experiment III.) Average for two Guernseys and two Jersey cows by five day periods. Linseed oil replacing starch in ration. Energy intake kept constant.

receiving 0.25 pound and 0.50 pound of linseed oil daily had a slightly lower butterfat percentage but higher milk and butterfat production than for the adjacent periods on the basal ration. She was the only exception even at the lower levels.

In general, the results of this experiment agreed with those of Experiment II, showing that even small increases in fat intake cause a slight increase in the fat content of the milk. With larger increases in the fat intake, the effect upon the fat percentage of the milk is greater.

Experiment IV

This experiment differed from the two preceding in that cocoanut oil, of a grade prepared for cooking purposes, was included in the ration at successive levels of 1.25, 0.75, and 0.25 pounds daily to each of six cows. It was not found possible at this time to select cows of uniform weight and production, consequently the relationship of fat intake to these two factors was not the same in all individuals. Two Holsteins, two Jerseys, and two Guernseys were used. As shown in figure 4, the butterfat test was greatly increased when 1.25 pounds of cocoanut oil were fed. Less marked increases appeared when smaller amounts of the oil were fed.

The most interesting point in the behavior of cocoanut oil was its effect upon milk yield. In every individual case, the milk yield was very definitely lowered during the period when 1.25 pounds of cocoanut oil were fed, with a recovery as soon as the cow was returned to the basal ration. The increased fat percentage of the milk was secured entirely through the lower milk yield, the butterfat yield remaining constant or being slightly lowered. When 0.75 or 0.25 pound of cocoanut oil was fed daily, the milk yield was not affected, the increased fat content being due to greater butterfat production. This was the only instance where the feeding of any of the oils or fats included in these studies showed any definite immediate effect upon milk yields.

Experiment V

This experiment was planned to determine the effect of soybean oil and peanut oil upon the fat content of the milk. Two Holstein cows, two Jerseys, and two Guernseys were included. The basal ration was the same as that used in Experiments II, III, and IV. Soybean and peanut oils (both of a grade suitable for cooking purposes) were fed, using the same plan as before with a period in which the basal ration was fed preceding and following each period of fat feeding. Each of the cows received 1.25 pounds of the oil daily. The energy intake was kept constant by decreasing the amount of starch in the ration when the fat was added.

The average results are shown in figure 4. Each individual responded in every case with an increased butterfat percentage when the oils were fed. The butterfat production was increased about 10 per cent. No uniform effect upon the milk yield was observed. In general, the effect of these two oils was quite similar to that of the fats studied in Experiment I.

Experiment VI

Throughout these experiments as well as in a previous study (1), it was observed that, when fats were added to the ration of cows, a definite interval of time elapsed before the effect was observed in the milk. Similar observations have also been reported by others. When fat was first added at

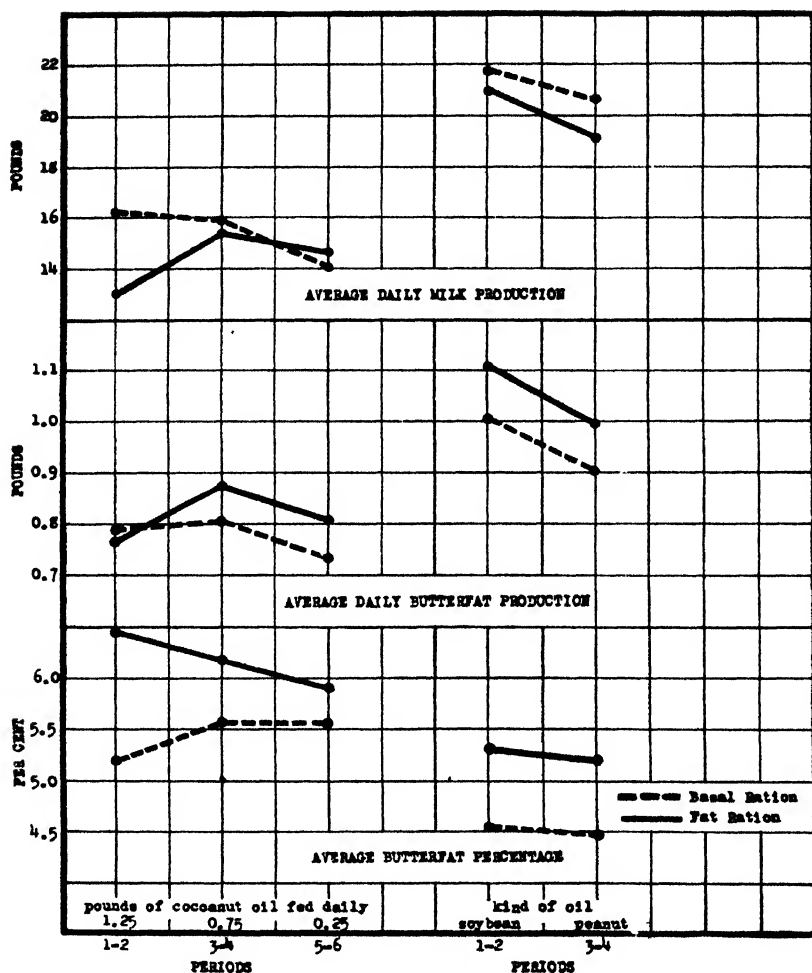


FIG. 4. MILK AND BUTTERFAT PRODUCTION AS INFLUENCED BY FEEDING DIFFERENT AMOUNTS OF COCOANUT OIL. (Experiment IV—left.) Average for two Holstein, two Guernsey, and two Jersey cows by five day periods. Coconut oil replacing starch. Energy intake kept constant.

MILK AND BUTTERFAT PRODUCTION AS INFLUENCED BY FEEDING 1.25 POUNDS DAILY OF SOY-BEAN OIL AND OF PEANUT OIL. (Experiment V—right.) Average for two Holstein, two Guernsey, and two Jersey cows by five day periods. Oils replacing starch. Energy intake kept constant.

the time of the evening milking, little change in the fat content of the milk was noted during the following day, but the full effect seemed to have appeared by the second day. Likewise, when the fat feeding was discontinued, the last fat being fed at the time of the morning milking, the fat content of the milk did not return to normal until the second day following the change. In all of these experiments the butterfat tests were made upon

composite samples, including both the morning and the evening milk. For this reason the duration of this lag in the effect of the fat could only be estimated within approximately 24 hours of the exact time. With the view of establishing this time more accurately, eleven different cows; including three Jerseys, three Guernseys, and five Holsteins; were milked at six hour intervals and each milking was tested separately.

The cows were fed the basal ration until they became thoroughly accustomed to the more frequent milking. Extreme care was observed to milk the cows entirely dry at each milking as incomplete removal of the milk from the udder is a very important factor in causing variations of the tests. Even with this precaution, the fluctuations are sufficient to introduce some confusion in determining the exact point at which the influence of the ingested fat first appears in the milk. In most cases, however, the change is sufficiently abrupt that it can be recognized readily when the fat percentage for each milking is compared with that of the corresponding milking on previous days.

Table 1 shows the results of these observations. The time at which the

TABLE 1

Time required after feeding butterfat for its influence upon the milk to be observed, and after removal from ration for its effect to disappear

(Experiment VI)

COW NO.	BREED	FIRST ADDITION OF FAT 0.35 POUNDS PER FEED OR 1.4 POUNDS DAILY		SECOND ADDITION OF FAT 0.35 POUNDS PER FEED OR 1.4 POUNDS DAILY		SINGLE FEED OF FAT 1.0 POUND
		Effect first observed after first feed of fat	Effect last observed after last feed of fat	Effect first observed after first feed of fat	Effect last observed after last feed of fat	Period of definite effect after feeding
		hours	hours	hours	hours	hours
Trial 1						
155	Jersey	18 to 24	30 to 36	12 to 18	doubtful	18 to 24
167	Jersey	12 to 18	30 to 36	18 to 24	30 to 36	24 to 30
190	Jersey	12 to 18	30 to 36	12 to 18	30 to 36	18 to 30
539	Guernsey	12 to 18	30 to 36	12 to 18	30 to 36	18 to 30
382	Holstein	18 to 24	24 to 30	24 to 30	30 to 36	18 to 24
402	Holstein	18 to 24	30 to 36	doubtful	36 to 42	24 to 30
411	Holstein	18 to 24	30 to 36	24 to 30	doubtful	doubtful
417	Holstein	18 to 24	30 to 36	18 to 24	doubtful	18 to 30
Trial 2						
167	Jersey	doubtful	36 to 42			12 to 18
190	Jersey	18 to 24	36 to 42			doubtful
411	Holstein	doubtful	doubtful			18 to 24
419	Holstein	doubtful	30 to 36			18 to 24
539	Guernsey	12 to 18	30 to 36			18 to 24
545	Guernsey	12 to 18	30 to 36			36 to 42
551	Guernsey	12 to 18	30 to 36			24 to 36

influence of the fat is first observed can be determined only within a range of six hours since the cows were milked at six hour intervals. The milk which is removed from the udder at the third milking after the change of feed is that which was produced between the twelfth and the eighteenth hours after the change. If this particular lot of milk is the first to show the influence of the addition of fat to the ration, it can only be said that the effect was not secured by the twelfth hour but had definitely appeared by the eighteenth hour. Similarly, when the fat is omitted from the ration, if the milk first fails to show the influence of the fat at the fifth milking after the change, the effect must have disappeared by the time of the previous milking or within 24 hours after the fat intake was lowered. This last milking to show the higher fat content having been produced between the eighteenth and the twenty-fourth hours, it may be assumed that the effect of the fat was still present after eighteen hours but had vanished by the twenty-fourth hour.

In the cases recorded as doubtful, the exact point at which the effect appeared could not be accurately distinguished due to fluctuations in the fat percentage. In every case, however, the influence of the change of feed could be unmistakably observed.

In trial 1, butterfat was added to the regular ration at the rate of 0.35 pound per feed or 1.4 pounds daily during two periods with intervening periods on the basal ration. After recovery from the second period of high fat intake, a single feeding of 1.0 pound of butterfat was given.

In trial 2, 1.4 pounds of butterfat were substituted daily for starch, equivalent in energy value, during a single period. After time had been allowed for recovery on the basal ration, 1.0 pound of fat was given at a single feeding.

Evidently, at least 12 to 24 hours, or perhaps on the average about 18 hours from the time of ingestion of fat, is required for the digestive and circulatory systems to place the first of this fat at the disposal of the mammary gland and for the gland to utilize it in the formation of milk fat, and about another 12 hours is required for the completion of the digestion and utilization of the ingested fat by the cows. The effect of a single heavy feed of fat appeared in about the expected time and the maximum effect persisted during only one or two milkings.

In general, the Holstein cows appeared to be somewhat slower in their response to the feeding of fat than were the Guernseys or Jerseys, but recognizing the limitations of the method as to the accuracy with which the first appearance of the effect could be observed, this can not be stated as a definite fact.

DISCUSSION

The almost perfect consistency of the results of these experiments can leave little doubt that the increased butterfat content of milk secured by

feeding fats to dairy cows is the effect which is characteristic of such fats.

During the course of these experiments and those previously reported (1), thirty different cows, including six Guernseys, ten Jerseys, and fourteen Holsteins, were used. In only one case, of the almost two hundred times that the fat intake of individual cows was increased by more than one-half pound daily, was there a failure to respond with an unmistakable increase in the fat content of the milk. This single exception was the Holstein cow reported under Experiment 1.

Butterfat, lard, tallow, corn oil, cottonseed oil, linseed oil, soy-bean oil, peanut oil, and cocoanut oil, when fed to adequately nourished lactating cows, were found to cause an increase in the fat content of the milk. This effect was secured either by adding the fat to an already adequate ration or by substituting the fat for carbohydrates of equivalent energy value. Increasing the energy intake by adding carbohydrates in the form of sugar to a ration already meeting the nutrient requirements of the cow did not influence the milk or butterfat production, nor the fat content of the milk.

It appears, therefore, that the increase in the fat content of milk secured by feeding fat is due to properties of the fat which render it more suitable material for milk fat production than other nutrients. The digested fat, in whatever form it may be made available to the mammary gland, is apparently particularly well adapted to the synthesis of milk fat, although unquestionably milk fat may be synthesized from carbohydrates or proteins of the ration, either directly or through the medium of body fat. That the food fat actually supplies the material from which at least part of the additional butterfat is produced is evidenced by the fact that the butterfat may take on properties characteristic of the fat which is fed.

The influence of the food fats is exerted regardless of the breed of the cow, stage of lactation, level of production, or season of the year.

The extent to which the fat percentage is affected is to a large degree proportional to the amount of fat which is fed. No evidence was observed that would indicate any depressing effect upon the fat content of the milk due to feeding large amounts of fat, although no attempt was made to determine the absolute maximum amounts which could be fed with favorable results. All of the fats were fed in amounts as high as 1.25 pounds daily and as much as 2.0 pounds daily were fed in some cases. This is far above the level that would be reached ordinarily with practical feedstuffs.

It is difficult to determine the minimum amount of added food fat which will influence the fat content of the milk. The fat percentage of the milk is subject to considerable fluctuation even though the ration is unchanged. This may be overcome partially by regularity in the time of milking and by care in removing all of the milk from the udder each time the cow is milked. Even with these precautions a certain amount of fluctuation is unavoidable. When dealing with small increases in the fat intake, the effect

is within the range of these fluctuations and consequently the results, particularly with individual cows, are likely to be inconsistent and difficult to interpret. The fluctuations in some cases will compensate for the influence of the feed while in other cases they may accentuate it. Possibly even very small increases in the fat intake may exert some influence, but inability to eliminate all other factors limits the ability to observe such effects. As soon as the increase in fat intake is sufficient so that its effect is greater than the range of the ordinary fluctuations, the results become consistent and are clearly observed. Many of the investigations upon this question have involved increases in the fat intake which were not sufficient to give clear cut results. This factor has probably been responsible for much of the disagreement in the conclusions reached by different investigators.

In addition to these random fluctuations of individual cows, unknown factors are encountered, which probably are connected with atmospheric conditions, and which frequently cause the fat percentage for entire herds of cows to rise or fall. An example of such a case is seen in Experiment I in which all of the cows had a considerably lower butterfat percentage during the mid-portion of the experiment than at the beginning or the end. Without proper control groups as a basis for comparison, such fluctuations as this might easily obscure the results, even when large amounts of fat are fed to groups containing several animals.

It could not be concluded on the basis of these experiments that any one of the fats included is more effective than the others as their behavior was quite similar. The only notable exception is cocoanut oil, which, when fed at the rate of 1.25 pounds daily, apparently caused a marked decrease in milk production. The butterfat production was maintained, however, and consequently the milk had a much higher fat content. When fed at the rate of 0.75 pound or less daily, the cocoanut oil did not seem to influence the milk yield but caused an increase in butterfat yield and consequently in the fat content of the milk.

In all cases, except the one noted when the largest amount of cocoanut oil was fed, the increased butterfat percentage appeared to be secured entirely through increased butterfat production. This is a very significant fact since an increase in fat percentage may be secured to an equal degree by a decrease in milk yield, the fat yield remaining constant or being reduced to a lesser extent. It is doubtful if it is ever justifiable to consider the fat percentage alone since the fat percentage is merely an expression of the relationship between the amount of milk produced and the amount of fat produced. If fat percentage and milk yield are considered as the characters which are controlled or influenced by factors of heredity or environment, then it must be assumed that butterfat yield is merely a relationship between the fat percentage and the milk yield. It seems much

more plausible and in better keeping with the present knowledge of the physiology of milk secretion to think of milk yield and butterfat yield as being the characters which are influenced directly, the fat percentage being merely a relationship which may be changed by an increase or decrease in either milk yield or in butterfat yield without a corresponding change in the other.

It has been generally observed that the secretion of fat in the mammary gland is to a certain degree independent of the secretion of the plasma portion of the milk. It is to be expected, however, that the relationship of the fat to the non-fat portion of the milk, expressed as fat percentage, will remain relatively constant, since factors such as the stimulus of hormones which influence the degree of activity of the gland will to a large extent affect both alike. In some cases, however, it may be possible to influence one without a corresponding influence upon the other.

When cows in a very fat condition are underfed and thus forced to draw upon their reserve food supply, consisting largely of fat, it has been shown by Eckles and Palmer (2) that the fat content of the milk is increased. Presumably this is due to the fact that this reserve, when returned to the blood stream, provides the mammary gland with material particularly well adapted to production of milk fat. If the cows are in advanced stages of lactation, this is accompanied by a rapid decline in milk production. The decline in butterfat production is less rapid, probably because the reserve of fat producing materials is more adequate than the reserve of materials such as protein which are necessary for the production of milk. Care was observed in this experiment to provide ample nutrients for the cows at all times by feeding slightly above their requirements, thus eliminating underfeeding as a possible factor in the results. The only case of underfeeding encountered was that of a heavily producing Holstein cow, which was unable to consume sufficient feed to satisfy her nutrient requirements. This cow, although she was underfed and drawing extensively upon her body reserves as indicated by a rapid loss of weight, responded to increased fat intake with a very marked increase in butterfat yield and butterfat percentage. Since the underfeeding may have been a contributing factor, the data for this cow were not included when the results of this particular experiment were summarized, but her behavior suggests that underfeeding at least does not seriously interfere with the response to increased fat intake.

The increase in butterfat production due to feeding fats was usually equivalent to but 10 to 20 per cent of the additional fat which was fed. It is hardly to be expected that the returns would be much greater when it is considered that the mammary gland merely receives an unselected portion of the blood supply and that any nutrients which may enter the blood stream are available, not only to the mammary gland, but at the same time

to all other parts of the body as well. In utilizing this alimentary fat the mammary gland is thus placed in competition with the other parts of the body where this material may be utilized as a source of energy or for storage as body fat. It is possible that the condition of the animal or the plane of nutrition might be factors which would influence the relative amount of the alimentary fat which would be utilized by the mammary gland for production of milk fat.

There appears to be no reason to believe that, when the fat intake is increased, the resulting increase in butterfat content of the milk is due to any cause other than a specific influence of the fats themselves. It seems most probable that this is due to the fact that this alimentary fat supplies to the mammary gland material which is particularly well adapted to the production of milk fat.

The delay in the response to fat feeding and the residual effect after the fat feeding is discontinued are probably due merely to the time required for the digestive and circulatory organs to place this material at the disposal of the mammary gland and for the secretory process to take place. When the fat intake is reduced to normal after a period of high fat intake, the butterfat production merely returns to normal and gives no evidence of being depressed to a subnormal level as would be likely to happen following an increased fat production due merely to a physiological upset or to a purely stimulative action comparable to that of drugs. There was no outward evidence of any digestive disturbance or physical disorder of any kind with the changes in fat intake.

These experiments were planned primarily to study the immediate effect of changes in fat intake upon the fat content of the milk. The six day periods were entirely satisfactory for this purpose but are inadequate for observations upon the influence of continued high fat intake. Experiment I, however, presents an interesting case in which a group of cows received a high fat intake during alternate periods including more than one-third of their entire lactation period. During this entire experimental period, considered as a whole, the average butterfat content of the milk of every cow was considerably above normal due to the fact that it was markedly increased during the periods of high fat intake, which constituted one-half of the total time. It is difficult to reconcile these facts and the continued response to the changes of fat intake with the generally accepted belief that the fat percentage will soon return to normal with continued fat feeding. It is doubtful if this has been adequately proven experimentally, and it is hoped that more satisfactory evidence will be secured from experiments now under way covering entire lactation periods.

SUMMARY

1. The fat content of the milk of dairy cows was markedly increased when the fat content of the ration was increased during six day periods by

feeding butterfat, lard, tallow, linseed oil, cottonseed oil, corn oil, peanut oil, soy-bean oil, or cocoanut oil.

2. The degree to which the fat percentage of the milk was influenced was, to a large extent, proportional to the amount of fat which was fed.

3. The increase in the fat content of the milk was secured regardless of the breed of the cows, stage of lactation, level of production, or season of the year.

4. The increase in fat percentage was due primarily to increased butterfat production, since the milk yield was influenced only to a slight extent, except in the case of cocoanut oil which appeared to cause a depression of milk yield when fed in large amounts.

5. The increased amount of butterfat in the milk was equivalent to but 10 to 20 per cent of the increase in fat intake, the remainder presumably being utilized by the body for other purposes.

6. A period of 12 to 24 hours after the fat was fed elapsed before its influence became observable in the milk. This influence was maintained for 30 to 42 hours after the last fat was fed. This lag apparently corresponds to the time required for the necessary digestive, circulatory, and secretory processes to take place.

7. The influence of the fat was exerted whether it was added to an already adequate ration or whether it replaced an equivalent amount of energy in the form of carbohydrates.

8. It was not found necessary to feed the fat in an emulsified form as it was very effective when fed after melting and mixing with the grain.

9. These investigations give no satisfactory evidence upon the effect of continued fat feeding because of the short experimental periods used.

REFERENCES

- (1) ALLEN, N. N. The fat percentage of milk as affected by feeding milk to dairy cows. *JOUR. DAIRY SCI.*, 15: 132-141. 1932.
- (2) ECKLES, C. H., AND PALMER, L. S. Influence of plane of nutrition of the cow upon the composition and properties of milk and butterfat. Influence of underfeeding. *Mo. Agr. Expt. Sta. Res. Bul.* 25. 1916.
- (3) JORDAN, W. H., AND JENTER, C. G. The source of milk fat. *N. Y. Agr. Expt. Sta. Bul.* 132. 1897.
- (4) JORDAN, W. H., JENTER, C. G., AND FULLER, F. D. The food source of milk fat with studies on the nutrition of milch cows. *N. Y. Agr. Expt. Sta. Bul.* 197. 1901.
- (5) MAYNARD, L. A., AND McCAY, C. M. The influence of different levels of fat intake upon milk secretion. *N. Y. (Cornell) Agr. Expt. Sta. Bul.* 543. 1932.

BUTTER AS A SUBSTRATE FOR MOLD GROWTH¹

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Several hundred mold cultures have been collected in our laboratory from butter and from the equipment or materials used in its manufacture and packaging. It was considered advisable to determine the ability of these molds to develop on butter since it is conceivable that certain types might be commonly recovered which have no significance as factors in the deterioration of butter. In these studies, unsalted butter was employed as the substrate inasmuch as the experimental evidence indicates that it is much more favorable for mold growth than salted butter. High humidities and a plentiful air supply were provided, where possible, to encourage the development of the cultures. The temperature of storage was varied for the purpose of studying its effect upon the growth.

REVIEW OF LITERATURE

The literature regarding the presence and growth of molds in butter has been reviewed exhaustively by the senior author (23). Since 1929, a number of papers have appeared that throw additional light on this important problem.

Morgan (28) (29), Grimes, Kennelly and Cummins (11) (12), and Bisby, Jamieson and Timonin (3) have recently listed in some detail the species of fungi found in the butter of three widely separated countries of the world. Others who have reported similar observations are Boekhout and Van Beynum (4), Gross (14), Libbert (22), and Macy, Combs and Morrison (25). Grimes and his coworkers (6) (13) (20) have provided additional material on certain types of fungi recovered from butter. The importance of parchment paper as a source of infection has been emphasized by Arup (1), Greger (9) and R. Hansen (18). General information on the mold problem has been presented by Bøgh-Sørensen (5), A. P. Hansen (16) (17), Knudsen and Nielsen (21), and Sørensen (36), while studies of churn contamination and sanitation have been made by Hammer and Olson (15) (32), Macy, Combs and Morrison (25) (30), and Widen (43).

The growth of molds in butter, the factors which may influence such development and the effect which these fungi may have upon the butter,

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have been investigated by Arup and van B. Gilmour (2), Demeter and Maier (7) (8), Grimes (10), Gross (14), Knudsen and Nielson (21), Macy and his colleagues (23) (24) (26) (27), Morgan (28) (29), Nelson (31), Parfitt and Galema (34), Pokrowsky (35), van B. Gilmour and Cruess-Callaghan (37), Voitkevich (38) and Walts (39). Closely related laboratory studies have been made by Hermann (19), Parfitt (33) and White and Hood (40) (41) (42). The conclusions reached in all of these reports are in general agreement with observations previously made and with those reported in this paper.

PURPOSE OF STUDY

The purpose of this study was to determine (1) which species and genera of molds in the collection could utilize unsalted butter a substrate when favorable growth conditions were provided, (2) what effect the molds had upon the appearance of the butter, (3) the influence of cold storage conditions on the growth, and (4) the effect which low temperatures of storage exerted on the subsequent development when the butter was placed in a more favorable environment.

METHODS OF PROCEDURE

Cultures:

Three hundred and seventy-two mold cultures were available for these studies. They had been isolated originally from butter and equipment or materials used in the manufacture or packing of butter.

The following species were identified (the number of cultures of each in parentheses): *Acremoniella brevia* (1); *Acrostalagmus cinnabarinus* (4); *Alternaria humicola* (11), *Alternaria fasciculata* (1); *Aspergillus niger* (31), *A. flavus* (17), *A. fumigatus* (12), *A. versicolor* (4), *A. nidulans* (2), *A. terreus* (2), *A. candidus*, *A. luteo-niger*, *A. micro-virido-citrinus*, *A. sydowi* and *A. ustus* (one each); *Hormodendron cladosporioides* (15), *H. viride* (2), *H. hordei*, *H. nigrescens* and *H. olivaceum* (one each); *Monilia sitophila* (3), *M. brunnea* (2) and *M. geophila* (one); *Mucor sylvaticus* (10), *M. corticolus*, *M. plumbeus* and *M. racemosus* (one each); *Oospora lactis* (14); *Penicillium terrestre* (8), *P. viridicatum* (7), *P. marzensii* (4), *P. palitans* (4), *P. notatum* (3), *P. blakesleei* (2), *P. brevi-compactum* (2), *P. camemberti* (2), *P. solitum* (2), *P. adametzi*, *P. baiiolum*, *P. bialowiezense*, *P. bifforme*, *P. biourgeianum*, *P. brunneo-violaceum*, *P. chermesium*, *P. chrysogenum*, *P. cyclopium*, *P. fellutatum*, *P. frequentans*, *P. gilmanii*, *P. griseo-brunneum*, *P. griseo-fulvum*, *P. howardii*, *P. italicum*, *P. johannioli* Zal., *P. miczinskii*, *P. psittacinus*, *P. puberulum*, *P. roqueforti*, *P. spinulosum*, *P. stoloniferum*, *P. suavolens*, *P. tabacinum*, *P. trzebinskii* and *P. urticae* (one each); *Rhizopus nigricans* (4), *R. speciosus* (one); *Spicaria divaricata* (3); *Trichoderma lignorum* (8) and *Trichothecium*

roseum (2). The remainder of the known genera were unidentified as to species. Thirty-one cultures remained unknown.

Preparation of butter:

Sweet cream (testing 28–33 per cent fat) was autoclaved for 20 minutes at 15 pounds pressure, after which it was cooled and held at 5° C. until ready for churning. The butter was made in sterile Dazey churns, washed with sterile water and worked under aseptic conditions. The composition of the butters ranged from 14 to 17.6 per cent moisture and from 0.6 to 3.0 per cent curd as determined by the Kohman method. Small cubes (1 sq. inch) of this unsalted butter were then placed in sterile petri dishes. The lid of each dish was held slightly raised by means of a small wire staple to allow access of air. The dishes containing the samples were placed immediately at 5° C. and allowed to stand overnight for hardening.

Inoculation:

The following morning the butter was inoculated by adding the culture to the small droplets of moisture which appeared on the surface of the chilled butter, and which provided an immediately available supply of moisture for spore germination. Care was taken to avoid transferring any of the culture medium to the butter. Triplicate plates were prepared for each culture.

Storage:

Immediately after the butters were inoculated they were placed in storage as follows: one set of plates at 20–23° C., another set at 4–6° C. and the third at –16 to –18° C. (For convenience, in later discussion, the storage temperature will be stated as 22° C., 5° C. and –18° C.) The samples were stored in twenty-gallon earthenware crocks provided with covers and false bottoms. In the crocks stored at 22° C. and at 5° C., water containing 1 per cent HgCl_2 was always kept under the false bottoms to provide for constant humidity and the preservative to maintain the water in a sterile condition. The relative humidity in the crocks at 20–22° C. ranged from 90 to 95 per cent, while at 4–6° C. it ranged from 73 to 83 per cent.

Observations:

Observations were made after one week and again after two weeks in the case of butters stored at 22° C. The butters held at 5° were examined after two and four weeks' storage, after which they were placed at 22° C. for observation after an additional two weeks. The butters kept at –18° C. were examined after twenty weeks, then placed at 5° C. for observation after another week, after which they were stored at 22° C. for one week at the end of which time they were examined again. Observations were made in each case by naked eye and at 50× magnification on the extent of growth,

the presence or absence of fruiting bodies, and the appearance of the butter. The odor was also noted.

PRESENTATION OF DATA

The results of these studies are summarized in tables 1 and 2.

Growth at 22° C.

The growth of the cultures at 22° C. was good or abundant in 216 out of the 372 cultures used. Only four cultures definitely failed to show visible signs of development. Five other samples were contaminated in such a manner that an accurate reading could not be made. These results demonstrate that the growth of molds on unsalted butter is not restricted to any appreciable extent because of a lack of suitable food supply. Where cultures failed to make visible growth, there might be microscopic evidence or factors other than nutriment may have been operative.

Among the species which failed to show visible growth at 22° C. were one of *Aspergillus niger*, two of *Oospora lactis* and one unidentified culture. It has been noted, however, in other studies that *O. lactis* may produce an extensive mycelium in butter without giving any visible signs of such activity.

Growth at 5° C.

In contrast with the results at 22° C. it was found that none of the cultures grew well at 5° C. during a two week period. Table 1 shows that more than one-third of them failed to give any visible signs of growth. These results indicate the effect of lower temperatures in the growth of molds. Even when the storage period had been prolonged to four weeks no further changes were noted. *Aspergillus* cultures seemed to be much more sensitive than those of *Penicillium*. This observation has been confirmed by one of the authors (23) in a previous study. Among the identified species, *Acremoniella brevia*, *Acrostalagmus cinnabarinus*, *Aspergillus candidus*, *Aspergillus micro-virido-citrinum*, *Aspergillus ustus*, *Monilia geophila*, *Mucor plumbeus*, *Penicillium fellutatum* and *Rhizopus speciosus* did not, in any culture of such species, show signs of growth at 5° C. after two weeks' incubation.

When the butter was taken from the 5° C. storage and placed as 22° C. for two weeks, most of the cultures, which did not exhibit visible growth at 5° C., began to develop, some of them abundantly as indicated in table 1. This noticeable effect of temperature is worthy of consideration, especially in connection with the implications concerning the commercial handling of butter. The lower temperatures were not fatal to the mold inocula but principally impeded the germination and further development.

Growth at -18° C.

Table 2 gives the results of studies at cold storage temperatures. During twenty weeks' incubation at -18° C. only two cultures, one of *Penicil-*

lium viridicatum and one of *Penicillium griseo-fulvum* gave any suggestion of development and this was so questionable that the appearance might have been due to mycelial threads carried over with the inoculum. The protective effect of low temperatures is clearly demonstrated.

When these samples were taken from the -18° C. storage, they were placed at 5° C. for a period of one week. No noticeable growth occurred during this time. After this exposure at 5° C. the butter was put at 22° C. for another week. As shown in table 2, most of the cultures recovered and developed reasonably well and in some cases as well as they had done at 22° C. without previous chilling or freezing. *Aspergillus* seemed to be retarded more than the other common forms. Many genera made perfect recoveries. Again *Oospora lactis* remained invisible in a number of instances.

TABLE 1

Extent of growth of molds on butter stored at various temperatures

GENUS	NUMBER OF CULTURES USED	EXTENT OF GROWTH OF CULTURES ON BUTTER AT											
		22° for 2 weeks				5° for 2 weeks				5° for 4 weeks, then 22° for 2 weeks			
		Abundant or good	Fair or poor	None visible	Discoloration of butter	Abundant or good	Fair or poor	None visible	Discoloration of butter	Abundant or good	Fair or poor	None visible	Discoloration of butter
Number of cultures													
Acremoniella	1	1	0	0	1	0	0	1	0	0	1	0	0
Acrostalagmus	6	0	6	0	3	0	2	4	0	0	1	2	0
Alternaria	19	8	11	0	19	0	18	1	0	11	7	0*	7
Aspergillus	95	62	30	1*	1	0	46	49	0	66	28	1	18
Botrytis	1	0	1	0	0	0	1	0	0	0	0	0*	0
Cephalosporium	3	1	2	0	2	0	2	1	0	2	1	0	1
Hormodendrum	19	7	12	0	15	0	16	3	0	4	13	0*	12
Monilia	8	0	6	0*	1	0	2	6	0	3	2	3	0
Mucor	13	6	7	0	8	0	6	7	0	8	3	0	0
Oospora	14	3	9	2	2	0	1	13	0	1	6	7	1
Penicillium	130	96	30	0*	50	0	98	32	0	99	30	0*	9
Phoma	4	2	2	0	3	0	3	1	0	1	3	0	4
Rhizopus	5	3	2	0	0	0	4	1	0	2	3	0	0
Spicaria	3	2	1	0	1	0	3	0	0	1	1	0*	1
Sporotrichum	1	1	0	0	1	0	0	1	0	0	1	0	1
Stemphylium	3	2	1	0	3	0	2	1	0	1	1	1	2
Syncephalastrum	5	5	0	0	0	0	5	0	0	5	0	0	0
Trichoderma	9	5	4	0	7	0	6	3	0	3	6	0	0
Trichothecium	2	0	2	0	1	0	2	0	0	0	2	0	1
Unidentified	31	12	17	1*	7	0	20	11	0	12	16	0*	7
Total	372	216	143	4	125	0	237	135	0	219	125	14	64
		363*				372				358*			

* Remainder of samples contaminated during storage.

Discoloration of butter:

Tables 1 and 2 indicate that none of the cultures caused any discoloration of the butter during storage at 5° C. or -18° C. This does not mean that the mold colonies which were visible failed to show any color. In fact, the colonies often were deeply pigmented in the hyphae or in the conidia but did not discolor the area beyond the mycelium. Wherever discoloration of the butter is indicated in the tables, it signifies that the butter

TABLE 2
Extent of growth of molds on butter stored at various temperatures

GENUS	NUMBER OF CULTURES	EXTENT OF GROWTH OF CULTURES ON BUTTER AT							
		- 18° C. for 20 weeks				- 18° C. for 20 weeks, then 5° C. for 1 week, then 22° C. for 1 week			
		Abundant or good	Fair or poor	None visible	Discoloration of butter	Abundant or good	Fair or poor	None visible	Discoloration of butter
Number of cultures									
Acremoniella	1	0	0	1	0	0	0	1	0
Acrostalagmus	6	0	0	6	0	0	6	0	0
Alternaria	19	0	0	19	0	2	9	1*	7
Aspergillus	95	0	0	95	0	40	44	11	9
Botrytis	1	0	0	1	0	0	1	0	0
Cephalosporium	3	0	0	3	0	0	3	0	0
Hormondendrum	19	0	0	19	0	1	7	2*	5
Monilia	8	0	0	8	0	2	2	2*	0
Mucor	13	0	0	13	0	5	6	0*	0
Oospora	14	0	0	14	0	0	8	5*	0
Penicillium	130	0	2	128	0	68	60	0*	18
Phoma	4	0	0	4	0	0	3	0*	3
Rhizopus	5	0	0	5	0	4	1	0*	0
Spicaria	3	0	0	3	0	1	2	0	0
Sporotrichum	1	0	0	11	0	0	1	0	1
Stemphylium	3	0	0	3	0	0	1	1*	1
Syncephalastrum	5	0	0	5	0	4	1	0	0
Trichoderma	9	0	0	9	0	5	3	1*	2
Trichothecium	2	0	0	2	0	0	1	0*	1
Unidentified	31	0	0	31	0	3	21	2*	1
Total	372	0	2	370	0	135	180	26	48
		372				341*			

* Remainder of samples contaminated during storage.

itself had been affected. The pigments often penetrated for considerable distances in the butter or spread completely through the surface areas. The colors were of many sorts and shades. They varied with the different species or genera and with the storage conditions. The discolorations pro-

duced by the various genera were as follows: *Acremoniella* and *Acrostalagmus*, brown; *Alternaria*, dark brown or black smudge, often involving the whole block of butter; *Aspergillus*, yellow dominant but also red, brown, green and black; *Cephalosporium*, brown to black; *Hormodendrum*, dark brown, dark green or black smudge often covering the cube of butter; *Monilia*, *Mucor*, yellow, brown or green; *Oospora*, red or brown; *Penicillium*, yellow or green dominant, orange, red, brown or black; *Phoma*, red, brown, green or black; *Spicaria*, orange or brown; *Sporotrichum*, black; *Stemphylium*, pink, red or reddish-brown; *Trichoderma*, yellow or black; *Trichothecium*, brown or black; and among the unidentified cultures, a variety of red, pink, yellow, orange, green, brown, or black. *Alternaria*, *Hormodendrum*, *Phoma* and *Stemphylium* produced the most extensive discoloration.

Aspergillus flavus, *A. micro-virido-citrinum*, *A. niger*, *A. sydowi*, *Penicillium batiolum*, *P. biforme*, *P. brunneo-violaceum*, *P. chermesium*, *P. chrysogenum*, *P. cyclopium*, *P. gilmanii*, *P. howardii*, *P. johannii* Zal., *P. miczinski*, *P. puberulum*, *P. suaveolens*, *P. trzebinskii*, *P. urticae*, *Monilia brunnea*, *Monilia sitophila*, *Mucor plumbeus*, *Mucor racemosus* and the cultures of *Botrytis*, *Rhizopus* and *Syncephalastrum* did not affect the color of the substrate by any penetration of the butter beyond the limits of the colony.

Effects on aroma of butter:

The most prominent defect in aroma which developed in the mold-inoculated butter was that described as "old cheese." Every genus, except *Botrytis*, contained species which produced such an odor. Other common criticisms were pungent, musty, fetid, volatile fatty acid, fruity, and unclean. *Aspergillus* cultures most often produced odors resembling the lower volatile fatty acids, while *Penicillium* cultures commonly brought about the development of pungent, peppery, fatty acid odors resembling those of Roquefort cheese. *Monilia*, *Mucor* and *Oospora* frequently were associated with fruity odors. *Alternaria*, *Hormodendrum* and *Phoma* were usually responsible for a musty or rather pungent, unclean, cheesy odor. There were instances where defects in odor were noticeable even where there were no visible signs of growth, and even at the lower temperatures of storage.

DISCUSSION OF RESULTS

The data presented substantiate previous work reported in the literature and indicate that most species of the common molds find unsalted butter a favorable substrate providing the environmental conditions are favorable.

It was shown that -18° C. for twenty weeks was sufficiently low to inhibit the growth of the molds studied on unsalted butter. Even though the molds failed to grow, there was some indication of a deleterious odor. This situation merits further investigation.

When the inoculated butters were removed from storage at -18° C. and held at higher humidities (73–83 per cent) for one week at 5° C., there was very little evidence of any germination of the molds. This agrees with previous reports by Morgan (28, 29) who found that at least two weeks were necessary for the growth of molds after the butter was defrosted. When the samples were placed at more favorable temperatures (22° C.), the molds developed as well as they did in a check group never subjected to low temperatures. This proves that the low temperature merely acted as a retarding influence and did not destroy the molds. If the cultures had been allowed to develop on the butter before cold storage, entirely different results might have been obtained, since germinating spores have been shown to be much less resistant to wide temperature variation than are the dormant conidia.

Butter is commonly held at 0° to 10° C. for short periods following cold storage. When the inoculated butters, that were stored originally at -18° C. for twenty weeks, were held at 5° C. for one week, this latter temperature was sufficiently low to prevent growth. However, when duplicate sets of seeded butters were placed directly into storage at 5° C. such a temperature was not low enough to check mold growth after four weeks and in many cases not after two weeks. Consequently, if unsalted butter is to be stored for two weeks or longer it must be kept below 5° C. to prevent the possible development of molds. As a matter of fact, one of the authors (23) found that certain species of *Penicillium*, *Hormodendrum* and *Alternaria* could grow at 0° C. so that temperatures below this would be advisable.

The discoloration due to mold development on the surface of butter, depreciates the market quality to such an extent that every producer seeks to prevent it. As shown in these studies, some molds have a tendency to produce marked pigmentation of the butter aside from the spotting effect due to the mold colony itself. The most marked effects were produced by cultures of *Alternaria*, *Hormodendrum*, *Phoma* and *Stemphylium*. The first two genera have consistently been reported as common causes of unsightly smudges on the surface of butter, and in these experiments proved to be the most important.

Most of the cultures studied were found to cause a noticeable change in the aroma of the butter. This phenomenon was not correlated with the extent of visible growth. Even at -18° C. such defects appeared. The odors in general were of two types, one which suggested the hydrolysis of fat and the other which indicated protein cleavage. There were combinations of the two decompositions which resulted in a variety of odors, many of which resembled those of old cheese. These results are likewise in accordance with observations made by other investigators.

From the evidence presented, it becomes evident that a large variety of

molds can grow on unsalted butter under favorable conditions. Defects in the appearance of the butter may occur and deleterious changes in the aroma may be expected wherever molds are able to develop, even slightly. Storage at 5° C. while inhibiting some molds, allowed many to germinate. Those species which caused the greatest defects at favorable temperatures were not seriously hampered at 5° C. A temperature of -18° C. prevented all visible growth but did not destroy the molds even after twenty weeks' exposure. Consequently, if butter is contaminated with molds, subsequent development of the molds may be expected to occur if conditions are favorable. In the case of unsalted butter, temperature would appear to be the most important controlling factor.

SUMMARY

1. Three hundred and seventy-two cultures of molds, representing nineteen known genera, seventy identified species, and thirty unidentified cultures, were used in this study. All of these cultures were originally isolated from butter and equipment or material used in its manufacture or packaging.

2. Small blocks of sterile, unsalted butter were inoculated with these cultures and stored at various temperatures to observe the extent of growth of the fungi.

3. The unsalted butter, kept under favorable conditions, definitely supported the growth of more than 96 per cent of the cultures.

4. Storage of inoculated butter at 5° C. for two weeks checked the growth of some of the molds.

5. No mold growth occurred on inoculated, unsalted butter during storage for twenty weeks at -18° C.

6. The low storage temperatures, 5° C. or -18° C., did not have any effect on the subsequent development of the cultures when favorable temperatures were later provided.

7. The appearance of the butter was marred appreciably by many of the cultures but most seriously by species of *Alternaria*, *Hormodendrum*, *Phoma* and *Stemphylium*.

8. The aroma of seeded butter was nearly always affected by the growth of the molds.

9. Unsalted butter must be kept at low temperatures if the growth of molds is to be prevented.

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REFERENCES

- (1) ARUP, P. The analysis and composition of vegetable parchment used for packing dairy products. *Analyst* **56**: 149-61. 1931.
- (2) ARUP, P. S., AND G. VAN B. GILMORE. Investigations to test bacteriologically and chemically the effect of cold storage on the keeping qualities of Irish Free State creamery butter. *Jour. Dept. Agr. Ireland* **31**: 179-89. 1932.
- (3) BISBY, G. R., M. C. JAMIESON AND M. TIMONIN. The fungi found in butter. *Canad. Jour. Research* **8**: 97-107. 1933.
- (4) BOEKHOUT, F. W. J., AND J. VAN REYNUM. Roode vlekken op beschimmelde boter. Verslag Ver. Exploit. Proefzuivelboerderij Hoorn 1930. pp. 1-11. 1931.
- (5) BØGH SØRENSEN, S. Skimmel i smør. *Mælkeritid.* **46**: 940-1. 1933.
- (6) CUMMINS, H. A., V. C. E. KENNELLY AND M. GRIMES. A study of fungi found in milk. *Sci. Proc. Roy. Dublin Soc.* **19** n. s.: 311-19. 1929.
- (7) DEMETER, K. J., AND F. X. MAIER. Vergleichende Untersuchungen über mikrobiologische Beschaffenheit und Qualitätsbefund von Sauerrahmbutter. *Milchw. Forsch.* **11**: 418-36, 437-84, 486-506, 507-18. 1931.
- (8) DEMETER, K. J., AND F. X. MAIER. Comparative investigations on the microbiological composition and the quality of butter. *Jour. Bact.* **21**: 41-2. 1931.
- (9) GREGER, J. Pergamentpapier als Träger von Schimmelpilzsporen. *Ztschr. Untersuch. Lebensmitl.* **63**: 560-64. 1932.
- (10) GRIMES, M. Microbiological analysis of butter in relation to flavor score. *Internatl. Dairy Congr. Eng. Ed.* 1931. (Sect. 2): 5-10. 1931.
- (11) GRIMES, M., V. C. E. KENNELLY AND H. A. CUMMINS. A study of fungi found in butter. *Sci. Proc. Roy. Dublin Soc.* **19** n. s.: 549-69. 1930.
- (12) GRIMES, M., H. A. CUMMINS AND V. C. E. KENNELLY. Etude des champignons trouvés dans le lait, la crème et le beurre. *Lait* **12**: 894-903, 1071-1079. 1932; **13**: 291-307. 1933.
- (13) GRIMES, M., M. O'CONNOR AND H. A. CUMMINS. A study of some *Phoma* species. *Brit. Mycol. Soc. Trans.* **17**: 97-111. 1932.
- (14) GROSS, M. In der Butter vorkommende Sprosspilze und deren Einwirkung auf die Butter. *Tartu Ülikooli Pümanduse Kabineti (Estonia) Teated* No. 3. 78 pp. 1933.
- (15) HAMMER, B. W., AND H. C. OLSON. Bacteriology of butter. III. A method for studying the contamination from churns. *Iowa Agr. Expt. Sta. Res. Bul.* **141**. 1931.
- (16) HANSEN, A. P. Lidt om Mug i Smør. *Mælkeritid.* **46**: 89-90. 1933.
- (17) HANSEN, A. P. Luftinfektion. *Mælkeritid.* **46**: 769-70. 1933.
- (18) HANSEN, R. Pergament. *Mælkeritid.* **44**: 786. 1931.
- (19) HERMANN, L. Betriebswirtschaftliche, chemische und bakteriologische Studien über Vorbuch.- und Molkenbutter. *Jahrb. d. Milchw.* **4**: 1-87. 1930.
- (20) KENNELLY, V. C. E., AND M. GRIMES. *Paezilomyces hibernicus*—a new species. *Sci. Proc. Roy. Dublin Soc.* **19** n. s.: 513-16. 1930.
- (21) KNUDSEN, S., AND S. NIELSEN. Nogle Jagtagelsar angaaende Skimmelvekst (Mug) paa Smør. *Mælkeritid.* **45**: 495-6. 1932.
- (22) LIBBERT, M. S. Churn contamination and methods of control. *Arkansas Agr. Expt. Sta. Bul.* **257**, pp. 49-50. 1930.
- (23) MACY, H. Some of the factors influencing the growth of molds in butter. *Minn. Agr. Expt. Sta. Tech. Bul.* **64**. 1929.
- (24) MACY, H. Quantitative changes in the microflora of butter during storage. *Jour. Dairy Sci.* **13**: 266-72. 1930.

- (25) MACY, H., W. B. COMBS AND H. B. MORRISON, JR. The churn as a source of molds in butter. *JOUR. DAIRY SCI.* 14: 398-403. 1931.
- (26) MACY, H., S. T. COULTER AND W. B. COMBS. Observations on the quantitative changes in the microflora during the manufacture and storage of butter. *Minn. Agr. Expt. Sta. Tech. Bul.* 82. 1932.
- (27) MACY, H., AND H. B. RICHIE. The mold and yeast count as an index of the keeping quality of butter. *JOUR. DAIRY SCI.* 12: 351-66. 1929.
- (28) MORGAN, G. F. V. Moulds in unsalted butter. *New Zeal. Jour. Agr.* 39: 38-46, 103-13, 174-79. 1929.
- (29) MORGAN, G. F. V. Moulds in unsalted butter. *New Zeal. Dept. Agr. Bul.* 149. 1930.
- (30) MORRISON, H. B., JR., H. MACY AND W. B. COMBS. Preliminary studies of churn sanitation. *JOUR. DAIRY SCI.* 14: 404-15. 1931.
- (31) NELSON, J. A. The correlation between the organisms found microscopically and the bacteriological deterioration of butter. *Montana Agr. Expt. Sta. Bul.* 267. 1932.
- (32) OLSON, H. C., AND B. W. HAMMER. Bacteriology of butter. V. Studies on the microorganisms in churns. *Iowa Agr. Expt. Sta. Res. Bul.* 159. 1933.
- (33) PARFITT, E. H. The influence of media upon the yeast and mold count of butter. *JOUR. OF DAIRY SCI.* 16: 141-47. 1933.
- (34) PARFITT, E. H., AND M. L. GALEMA. Influence of air upon cream deterioration. *Natl. Butter Jour.* 22 (No. 6): 13, 14, 40. 1931.
- (35) POKROWSKY, W. L. Experimental researches on the effect of the temperature of storage of butter on the development of mold. (Translated title) *Kholodilnoie Dielo*, nos. 5-6. pp. 18-19. 1927. (Original in Russian seen. Translation by Dr. C. Nikiforoff).
- (36) SØRENSEN, H. A. *Mug. Mælkeritid.* 45: 958-60. 1932.
- (37) VAN B. GILMOUR, G., AND G. CRUESS CALLAGHAN. Rate of growth of microorganisms in Irish Free State creamery butter. *Jour. Dept. Agr. Ireland* 31: 226-31. 1932.
- (38) VOITKEVICH, A. F. Mold on butter and the methods of combating it. *Jour. All-Russ. Congr. Bot., Leningrad.* 1928: 201. 1928. Abstract in *Biol. Abstracts* 5: 1749. 1931.
- (39) WATTS, C. C. The action of *Penicillium roqueforti* on butter. *Arkansas Agr. Expt. Sta. Bul.* 257. pp. 51-2. 1930.
- (40) WHITE, A. H., AND E. G. HOOD. A study of methods for determining numbers of moulds and yeasts in butter. *Internatl. Dairy Congr. Eng. Ed.* 1931. (Sect. 2): 20-43. 1931.
- (41) WHITE, A. H., AND E. G. HOOD. A study of methods for determining numbers of moulds and yeasts in butter. I. The relation of the pH of the medium. *JOUR. DAIRY SCI.* 14: 463-76. 1931.
- (42) WHITE, A. H., AND E. G. HOOD. A study of methods for determining numbers of moulds on yeasts in butter. II. The influence of temperature and time of incubation. *JOUR. DAIRY SCI.* 14: 494-507. 1931.
- (43) WIDEN, E. Om jästsvamparnas inverkan på smörets smak. *Svenska Mejeritid.* 23: 295-98. 1931.

THE FEEDING VALUE OF ARTIFICIALLY DRIED PASTURE HERBAGE FOR MILK PRODUCTION

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The nutritive value of artificially dried pasture grass is the subject of considerable investigation. Hodgson and Knott (1) conducted digestibility trials with dairy heifers and found artificially dried pasture grass to be highly nutritious. The crude protein which constituted 24.64 per cent of the dry matter, was 74.92 per cent digestible. The coefficients of digestibility of the dry matter, the crude fiber and nitrogen-free extract were 67.57, 72.68 and 74.55 per cent, respectively. The average digestibility of the ether extract was 21.90 per cent. Newlander and Jones (2) reported the following coefficients of digestibility for artificially dried pasture grass: dry matter 72.4 per cent; crude protein, 71.0 per cent; crude fiber, 73.3 per cent; nitrogen-free extract, 81.6 per cent; and ether extract, 46.4 per cent.

Newlander (3) conducted feeding trials using the reversal method with two groups of five cows each in comparing the feeding value of dried grass with a grain mixture. One group was fed mixed clover and timothy hay, corn silage and a grain mixture, while the test group received dried grass in place of the grain ration. He found that "These trials showed that grasses cut at intervals of from seven to 10 days resembles closely in composition and digestibility the 20 per cent dairy ration used. The fiber content of the dried grass was somewhat higher and the nitrogen-free extract content correspondingly lower. When 10 lbs. of the artificially dried grass was fed with liberal allowances of hay and silage and a small amount of grain, entirely satisfactory results were secured. Feeding this grass with two-thirds the regular allowance of silage and hay and no grain produced excellent results. While average milk production could be obtained by feeding this grass instead of all purchased concentrates, it was deemed advisable to supply about one-third the usual amount of grain when maximum production was desired or when high producing cows are being fed."

Watson (4) found artificially dried grass very palatable to lactating dairy cows. He reported that when cows were receiving dried grass as the major part of the ration milk production was efficiently maintained.

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Following the digestion and mineral balance studies reported by Hodgson and Knott (1) two feeding trials have been conducted with lactating dairy cows to determine the possibility of using artificially dried pasture herbage in place of a part or all of the concentrate mixture. These experiments are not as complete as is to be desired, but it is hoped that it will be possible to conduct further investigations of a more comprehensive nature at a later date.

EXPERIMENT I.³ THE VALUE OF ARTIFICIALLY DRIED PASTURE GRASS FOR
MILK PRODUCTION WHEN FED AS TWENTY PER CENT
OF THE GRAIN MIXTURE

Experimental

The artificially dried pasture herbage⁴ was the same as that used in the digestion experiment previously reported (1). It was grown at the Western Washington Experiment Station and contained a mixture of English ryegrass (*Lolium perenne*), Italian ryegrass (*L. italicum*), Orchard grass (*Dactylis glomerata*), rough stalk meadow grass (*Poa trivialis*), Kentucky bluegrass (*P. pretensis*), annual bluegrass (*P. annua*), meadow fescue (*Festuca elatior*), creeping bent (*Agrostis stolonifera*), Ladino clover (*Trifolium repens latum*), Alsike clover (*T. hybridum*) and white clover (*T. repens*). The predominating plants in this mixture were English ryegrass and Italian ryegrass. The herbage was cut at 14-day intervals and dried immediately after cutting. The material was dried in a home-made oven drier where hot air, from gas, was used as a source of heat. The hot air passing through the oven at about 150° F. dried the material to approximately five per cent moisture in from 10 to 12 hours. The dried herbage retained its green color and possessed a pleasing aroma. It was cut into approximately three-inch lengths and mixed with the grain ration. Table 1 gives the percentage of ingredients in the concentrate mixtures.

The experiment was conducted by the double reversal method with preliminary periods of one week and periods of comparison three weeks in

TABLE 1
Ingredients in concentrate mixtures expressed in percentages

MIXTURE	GROUND BARLEY	GROUND OATS	WHEAT BRAN	LINSEED MEAL	DRIED GRASS	STERILIZED BONE FLOUR	SALT
Basal mixture	45	20	27	5		2	1
Experimental mixture	45	20	12		20	2	1

³ This experiment was conducted at the Washington Agricultural Experiment Station, Pullman.

⁴ The writers are indebted to M. S. Grunder, Agronomist, Western Washington Experiment Station, Puyallup, under whose direction the herbage was collected and dried, and who devised the small experimental drier.

length. Two groups of four producing Holstein cows that were as nearly alike as possible with regard to age, live weight, production, periods of gestation and lactation, health and condition, were used. One group was fed the basal concentrate mixture, while the other group received the mixture containing the grass in place of the basal concentrate mixture. During this experiment, both groups received No. 1 chopped alfalfa hay fed at the rate of $1\frac{1}{2}$ pounds daily per 100 pounds live weight, and good quality sunflower silage fed at the rate of 2 pounds daily for each 100 pounds of live weight. The concentrate mixtures were fed at the rate of 2 pounds daily for each 5 pounds of milk produced over 10 pounds per cow. The same amount of hay, silage and grain was fed both groups with adjustments within each group to meet individual requirements. All feeds were carefully sampled for chemical analysis. Refused feed was weighed and analyzed.

Live weights of each cow were obtained on three successive days, the last two days of each period and the first day of the succeeding period. The milk was weighed each milking; aliquot samples taken and butterfat determinations were made weekly from the composite samples.

Results

The chemical composition of the experimental feeds is given in Table 2. The digestible crude protein content was 9.0 per cent of the basal mixture, and 9.1 per cent of the experimental mixture. The total digestible nutrient

TABLE 2
Percentage composition of experimental feeds

INGREDIENT	DRY MATTER	CRUDE PROTEIN	CRUDE FIBER	NITROGEN-FREE EXTRACT	ETHER EXTRACT	ASH
Alfalfa hay	87.53	20.08	12.65	41.58	4.44	8.79
Sunflower silage	31.29	2.80	7.26	17.15	0.68	3.40
Barley	89.28	7.70	5.83	71.47	1.68	2.60
Oats	90.06	9.04	9.08	63.50	4.66	3.78
Bran	88.82	15.28	4.76	63.15	2.30	3.33
Linseed meal	90.40	30.41	6.59	46.58	1.13	5.69
Dried grass	86.05	21.20	15.57	36.65	3.01	9.62

TABLE 3
Average daily ration consumed, gain in live weight and production per cow

EXPERIMENTAL PERIOD	NO. OF COWS	CONCENTRATE MIXTURE	SILAGE	HAY	GAIN LIVE WEIGHT	MILK PRODUCTION	FAT PRODUCTION	PRODUCTION 4% FAT-CORRECTED MILK
		<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>
Basal	8	15.4	25.6	19.0	0.70	44.6	1.383	38.6
Experimental	8	14.3	25.3	19.2	0.42	43.5	1.357	37.8

content was 70.7 per cent and 69.2 per cent respectively of the basal and experimental rations. Table 3 shows the average daily ration consumed, gain in live weight and production per cow while on the basal and experimental mixtures. In table 4 are given the feed and total digestible nutri-

TABLE 4
Feed and total digestible nutrients consumed per unit of production

FEED CONSUMED FOR PRODUCTION OF	BASAL	EXPERIMENTAL
	<i>lbs.</i>	<i>lbs.</i>
100 pounds of milk:		
Concentrate mixture	34.5	32.9
Hay	42.6	44.1
Silage	57.4	58.2
Total digestible nutrients	53.6	52.9
One pound of butterfat:		
Concentrate mixture	11.1	10.5
Hay	13.7	14.1
Silage	18.5	18.6
Total digestible nutrients	17.2	16.9
100 pounds 4% fat-corrected milk:		
Concentrate mixture	39.9	37.8
Hay	49.2	50.8
Silage	66.3	66.9
Total digestible nutrients	61.9	60.8

ents consumed for the production of 100 pounds of milk, one pound of butterfat and 100 pounds of four per cent fat-corrected milk while on the basal and experimental rations.

The experimental grain ration, while very bulky, was palatable and was readily eaten. There was a slightly greater consumption of grain and silage by the cows while on the basal ration. There was also a greater gain in live weight and a slightly greater production of milk and butterfat when on the basal ration. The consumption of feed and total digestible nutrients per unit of production, however, was slightly in favor of the experimental mixture. The differences are so small that the results clearly indicate that artificially dried pasture herbage may be efficiently substituted for as much as 20 per cent of the concentrate mixture of a ration for producing cows.

EXPERIMENT II.⁵ ARTIFICIALLY DRIED PASTURE GRASS AND ALFALFA HAY *versus* ALFALFA HAY AS A RATION FOR DAIRY COWS

Experimental

The dried herbage used in this experiment was a mixture of English rye-grass (*Lolium perenne*), Italian ryegrass (*L. italicum*) and white clover

⁵ This experiment was conducted at the Western Washington Experiment Station, Puyallup.

(*Trifolium repens*). There was a considerable infestation of weeds in the field, that were cut and dried with the herbage. The material when cut represented herbage of two weeks' growth. Drying was accomplished in a small experimental, single drum, direct heating rotary drier. The grass passed through the drier in approximately 10 minutes and was dried at an outlet temperature of 310 to 320° F. At this temperature the grass was dried to an average moisture of 10 per cent. The herbage retained its green color and was palatable to the experimental animals.

The feeding trial was conducted with two groups of two cows each by the reversal method through two periods of comparison of four weeks each preceded by a preliminary period of one week. It is realized, of course, that the number of animals used was small, but the supply of artificially dried pasture grass available at that time was limited and it was thought advisable to use what was available. The two groups of cows were paired as nearly as possible with regard to periods of lactation and gestation, production, weight, age and condition. The cows were in the latter third of their lactation periods; therefore, high production did not prevail during the course of the experiment.

One group of cows was fed chopped No. 1 alfalfa hay, while the other was fed the same quality of hay plus 6.53 pounds daily of artificially dried pasture herbage. The hay was provided so that each cow had free access to it at all times. The dried grass was fed twice daily in limited amounts. The hay and the dried grass were sampled regularly for chemical analysis. Refused feed was weighed and analyzed.

Live weights of the cows were determined on three successive days at the beginning and once a week during each period. The milk was weighed each milking, aliquot samples taken and butterfat determination made from weekly composites. Water was available to the cows at all times and they were kept in comfortable box stalls during the entire experiment.

Results

Table 5 gives the percentage composition of the experimental feeds. In table 6 is given the feed consumption of the cows on the two types of rations. Table 7 shows the gain or loss in live weight and the production of milk,

TABLE 5
Percentage composition of experimental feeds

INGREDIENTS	DRY MATTER	CRUDE PROTEIN	CRUDE FIBER	ETHER EXTRACT	NITROGEN-FREE EXTRACT	ASH	TOTAL DIGESTIBLE NUTRIENTS
Alfalfa hay	88.0	14.84	24.96	1.68	37.56	8.96	49.75
Artificially dried pasture herbage	89.73	20.67	15.80	3.97	40.33	9.03	59.00

TABLE 6
Average ration consumed per cow

EXPERI- MENTAL PERIOD	NO. OF COWS	PERIOD I		PERIOD II				TOTAL DI- GESTIBLE NUTRI- ENTS
		Alfalfa hay		Alfalfa hay		Dried grass		
		Total	Av. daily	Total	Av. daily	Total	Av. daily	
Alfalfa hay	4	<i>lbs.</i> 3673.7	<i>lbs.</i> 32.28	<i>lbs.</i>	<i>lbs.</i> .	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i> 1827.70
Alfalfa hay and dried grass	4			3438.4	30.70	731.6	6.53	2142.64

TABLE 7
Average gain or loss in live weight and production per cow

EXPERI- MENTAL PERIOD	NO. OF COWS	GAIN OR LOSS IN LIVE WEIGHT		MILK PRODUCTION		FAT PRODUCTION		PRODUCTION 4% FAT-CORRECTED MILK	
		Total	Av. daily	Total	Av. daily	Total	Av. daily	Total	Av. daily
Alfalfa hay	4	lbs. -47.0	lbs. -0.42	lbs. 1455.1	lbs. 12.99	lbs. 52.49	lbs. 0.47	lbs. 1369.3	lbs. 12.23
Alfalfa hay plus dried grass	4	34.9	0.33	1711.3	15.28	59.75	0.53	1580.8	14.11

butterfat and four per cent fat-corrected milk for cows on the basal and experimental rations.

When the daily allowance of artificially dried pasture grass was limited to 6.53 pounds, it was readily eaten and proved to be very palatable. The average daily consumption of alfalfa while the cows were receiving the dried grass ration was less than when only alfalfa was given. However, the total digestible nutrient consumption was greater when they were receiving the grass and hay ration than when the alfalfa hay ration was fed. There was a larger production of milk and gain in live weight while the cows were receiving the ration of alfalfa hay plus dried grass.

The greater production and gain in live weight was sufficient to offset the larger consumption of nutrients. As indicated in table 8, the feed and total digestible nutrients consumed per unit of production was practically the same when the cows were receiving a diet of alfalfa hay and artificially dried pasture grass or the alfalfa ration. These data indicate that by affording dried grass in addition to alfalfa hay a larger consumption of feed

TABLE 8
Feed and total digestible nutrients consumed per unit of production

FEED CONSUMED FOR PRODUCTION OF	ALFALFA HAY PERIOD	ALFALFA HAY PLUS DRIED GRASS PERIOD
	<i>lbs.</i>	<i>lbs.</i>
100 pounds of milk:		
Alfalfa hay ..	252.47	200.92
Dried grass . .		42.74
Total digestible nutrients ..	125.60	125.18
One pound butterfat:		
Alfalfa hay	69.99	57.55
Dried grass		12.24
Total digestible nutrients	34.82	35.85
100 pounds 4% fat-corrected milk:		
Alfalfa hay	268.29	217.51
Dried grass		46.28
Total digestible nutrients	133.47	135.51

nutrients was obtained which resulted in a greater production, but the efficiency of production remained about the same.

SUMMARY

Two feeding trials were conducted in which artificially dried pasture herbage served as part or all of the concentrate mixture fed to dairy cows.

In the first experiment a concentrate mixture containing 20 per cent of artificially dried pasture grass was compared with a basal ration of similar composition.

The differences in production and gain in live weight of cows receiving the experimental ration as compared with the basal ration were too small to be significant. A slightly greater gain in live weight and production occurred when cows were receiving the basal ration. The feed consumption per unit of production was less for the cows on the experimental ration.

In the second experiment a ration consisting of alfalfa hay and artificially dried pasture grass was compared with a ration of alfalfa hay.

There was a greater consumption of the total digestible nutrients when cows were receiving the alfalfa hay and dried pasture grass ration. The larger consumption of digestible nutrients was accompanied by a greater gain in live weight and an increase in the production of milk and butterfat. The nutrients required per unit of production was approximately the same for both rations.

The results of these experiments indicate that artificially dried pasture herbage may be efficiently used for at least a part of the concentrate mixture of lactating cows.

REFERENCES

- (1) HODGSON, R. E., and KNOTT, J. C. Apparent digestibility of, and nitrogen, calcium, and phosphorus balance of dairy heifers on, artificially dried pasture herbage. *Jour. Agr. Res.* 45: 9. 1932.
- (2) NEWLANDER, J. A., and JONES, C. H. The digestibility of artificially dried grass. *Vt. Agr. Expt. Sta. Bul.* 348. 1932.
- (3) NEWLANDER, J. A. The feeding value of artificially dried young grass. *Vt. Agr. Exp. Sta. Bul.* 350. 1933.
- (4) WATSON, S. J. Investigations into the intensive system of grassland management. IV. The digestibility and feeding value of artificially dried grass. *Jour. Agr. Sci.* Vol. 21, Pt. 3. 1931.

American Dairy Science Association Announcements

HOUSING PLANS FOR THE 1934 MEETING

JUNE 26, 27, AND 28, 1934

ITHACA, NEW YORK

Willard Straight Hall, on the Cornell Campus, will be the social headquarters of the Association. The official registration desk will be in the lobby of this building. Meals may be obtained here. Rooms will be assigned from here, and the official programs will be distributed from the registration desk. All mail for visitors should be addressed in care of Willard Straight Hall.

Rooms. Good rooms will be provided in one of the University dormitories at \$2.00 per person per night. Persons staying three nights will be charged \$5.00, four nights, \$6.00. Cots will be provided for children in parents' room at \$1.00 per night.

It is very desirable that rooms be reserved in advance. Address E. S. Savage, Animal Husbandry Building, Ithaca, New York, and state carefully the number of persons in your party, men and women, and if provision should be made for any children.

A card will be sent giving the room number and name of the dormitory for all registrations received by June 10, 1934.

On arrival in Ithaca go directly to Willard Straight Hall and get your room assignment and program.

Please help the housing committee by *registering in advance*.

A. C. DAHLBERG

H. E. ROSS

E. S. SAVAGE, *Chairman*

MEETING OF THE SOUTHERN DIVISION

The Southern Division of the American Dairy Science Association held its annual meeting on January 31, February 1 and 2 in Memphis, Tennessee. There was an average attendance of about 25 at these sessions. Chairman A. D. Burke, of Alabama, presided. Abstracts of all papers have been prepared in a mimeographed volume.

Papers were presented on relation of dairying in Tennessee Valley to national dairy development by C. E. Wylie, on the influence of Mung Bean hay on fat percentage in milk by A. H. Kuhlman, on the influence of season of freshening on milk yields of Jersey cows by P. T. Dix Arnold and

R. B. Becker, on influence of season and advancing lactation on fat content of milk by R. B. Becker and P. T. Dix Arnold, on fly sprays by W. H. Eaton, on the feeding value of machine dried roughage by J. A. Sims, on trench silos by F. R. Edwards, on Korean Lespedeza hay by C. O. Jacobson, on grain feeding experiments by R. R. Graves, on fertilization of pastures by E. C. Elting and J. P. La Master, on temporary grazing crops by R. H. Lush, on dairy shows by Earl Weaver, on herd testing of college herds by L. O. Colebank, on cultured buttermilk from dry skim milk by A. D. Burke, on the composition of Oklahoma butter by E. L. Fouts, on the whipping of butter mixes by A. J. Gelpi, on extra heavy cream by J. R. Moss and B. E. Goodale, on applying a dairy code to a city milk supply by C. A. Hutton, and on a short course for milk inspectors by J. I. Keith.

The ballot by mail resulted in the election of R. H. Lush, of Louisiana, Chairman; E. C. Elting, of South Carolina, Vice-Chairman, and A. H. Kuhlman, of Oklahoma, as Secretary.

JOURNAL OF DAIRY SCIENCE

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STANDARDS AND METHODS OF ANALYSIS OF DRY SKIM MILK

E. C. THOMPSON, A. H. JOHNSON AND M. KLOSER*

The increased application and use of dry skim milk in various industries have invited the further consideration of the Standards Committee of the American Dry Milk Institute to review the original accepted standards and determine whether further changes could be made that would result in a better quality of product for human consumption and suitable standards be established for dry skim milk intended for animal feed.

To appreciate fully the changes outlined in this first revision of standards for grading dry skim milk, the reader is referred to those originally published in this JOURNAL, Volume XIII, No. 4, July, 1930.

GENERAL REQUIREMENTS FOR ALL GRADES FOR HUMAN CONSUMPTION

1. All dry skim milk for human consumption shall conform in all respects to Federal and State government regulations in force at the present time or that may be subsequently issued from time to time.

2. The factory and factory equipment used in the manufacture of dry skim milk shall be maintained in a strictly clean sanitary condition. No person or persons affected with any infectious, contagious or communicable disease, or who resides, boards or lodges in a household in which there is a person affected with such disease, shall be employed or permitted to work in or about any part of the factory in which dry skim milk is manufactured.

3. Dry skim milk shall be made from freshly skimmed milk to which no preservative, alkali, neutralizing agent, or other chemical has been added and which has been pasteurized in the liquid state either before or during the process of manufacture at a temperature of 142° F. for 30 minutes or its equivalent in bacterial destruction.

4. Dry skim milk shall be reasonably uniform in composition. The color shall be white or light cream and free from a brown or yellow color typical of overheated stock and free from any other unnatural color.

5. The flavor and odor of dry skim milk in powdered form or in re-solution shall be sweet and clean and entirely free from rancid, tallowy.

Received for publication January 11, 1934.

* The addresses of the authors are The Borden Company, 350 Madison Avenue, New York City, Research Laboratories of National Dairy Products, 1403 Eutaw Place, Baltimore, Md., and Bowman Dairy Company, 140 West Ontario St., Chicago, Ill.

fishy, cheesy, soapy, or other equally objectionable flavors or odors. It shall be substantially free from brown specks.

6. The package, preferably a barrel, drum or similar container with liner, shall be of such a character as to prevent contamination by dust, dirt, or other foreign matter and prevent the absorption of moisture and foreign odors.

SPECIFIC REQUIREMENTS FOR EXTRA GRADE

Extra Grade is so designated to indicate the highest quality of dry skim milk and, in addition to the above, must meet the following requirements:

	SPRAY NOT GREATER THAN	VACUUM DRUM NOT GREATER THAN	ATMOSPHERIC ROLLER NOT GREATER THAN
Butterfat	1.50%	1.50%	1.50%
Moisture	4.00%	4.00%	4.00%
Titratable Acidity (reconstituted basis)	.15%	.15%	.15%
Solubility Index	1.25 cc	2.00 cc	15.00 cc
Bacterial Count (reconstituted basis)	15,000 per cc	15,000 per cc	15,000 per cc
Sediment	Disc No. 3	Disc No. 3	Disc No. 3

The dry skim milk shall be entirely free from hard lumps. The powder, as well as the reconstituted skim milk shall be entirely free from any storage or scorched flavor or odor.

SPECIFIC REQUIREMENTS FOR STANDARD GRADE

Standard Grade includes all dry skim milk that fails in one or more particulars to meet the requirements of Extra Grade but it must also meet the following requirements:

	SPRAY NOT GREATER THAN	VACUUM DRUM NOT GREATER THAN	ATMOSPHERIC ROLLER NOT GREATER THAN
Butterfat	2.00%	2.00%	2.00%
Moisture	5.00%	5.00%	5.00%
Titratable Acidity (reconstituted basis)	.17%	.17%	.17%
Solubility Index	1.5 cc	5.0 cc	15.00 cc
Bacterial Count (reconstituted basis)	50,000 per cc	50,000 per cc	50,000 per cc
Sediment	Disc No. 4	Disc No. 4	Disc No. 4

The dry skim milk shall be reasonably free from hard lumps but may have a slight storage or slightly scorched flavor or odor before and after reconstitution.

SPECIFIC REQUIREMENTS FOR THIRD GRADE

Any dry skim milk failing in one or more particulars to meet the requirements for Standard Grade shall be classed as Third Grade. However, such powder showing any one of the following characteristics shall be deemed unfit for human consumption:

Moisture—over 5%	} On reconstituted basis
Bacterial count more than 100,000 per cc	
Acidity greater than .20%	
Sediment greater than Disc No. 5	

Flavor and odor indicative of neutralization, fermentation or decomposition.

It is generally agreed that the bacterial count of dry skim milk is not necessarily an indication of the quality of the raw milk from which the dry product is made. The process of manufacture has a direct bearing upon the extent to which the bacteria are destroyed.

METHODS OF ANALYSIS

For a discussion of the determinations made as a basis for the appraisal of quality and for the methods of analysis, the reader is referred to this JOURNAL, Vol. XIII, No. 4, July, 1930, pages 322–335. No changes are recommended in the methods of analysis with the exception of the following which have been approved by the American Dry Milk Institute.

1. *Moisture Determination.* On account of a number of changes that have been made in the technic of this determination the procedure is outlined completely in the following:

Apparatus required:

A Bidwell and Sterling moisture tester complete including a 300 cc Erlenmeyer flask, 2 one hole cork stoppers, distillation tube and condenser. (See Ind. Eng. Chem. Vol. 17, page 147.)

A Precision Electric Heater type RH with rheostat.

Rubber tubing suitable for water connections $1\frac{1}{4}$ " inside diameter.

Condenser brushes (bristle $\frac{1}{2}$ " diam. $3\frac{1}{2}$ " long, wire handle 24" long).

Tube brushes (brushes $\frac{1}{4}$ " diam. 4" long, wire handle 13" long).

Ring stands and clamps suitable for holding equipment.

Procedure. Transfer a 50 gram sample as quickly as possible to a clean, dry 300 cc Erlenmeyer flask. Immediately pour sufficient toluol into the flask to cover the sample. This requires about 75 to 100 cc. Connect the flask with the condenser by means of a distillation tube. When it is desired to start the distillation, fill the distillation tube with toluol by carefully pouring through the top of the condenser. Bring to a boil rapidly by turning the heater on full. Shake frequently so as to prevent the powder from burning on the bottom of the flask. Just as soon as boiling

has begun reduce the heat so that the toluol will condense into the distillation tube at the rate of about 4 drops per second.

Thirty (30) minutes after distillation has begun, dislodge any water particles in the condenser tube by means of a condenser brush and wash down with 10 cc of toluol. Twenty (20) minutes later repeat and continue the distillation for an additional ten (10) minutes. Again dislodge any water particles and wash down the condenser tube as before and note if there has been any increase in the moisture reading. If an increase has taken place, continue the distillation another 15 minutes and wash down as before. If no increase has taken place, the continued additional distillation period of fifteen (15) minutes will be unnecessary. Spray powders do not usually require the additional distillation while roller process powders do.

After the distillation tube has come to room temperature the large water particles in the toluol layer may be readily dislodged by means of a piece of wire, the finer particles by the use of a tube brush. Care should be exercised to make sure that the brush does not dip into the water layer while this is being done. An uneven meniscus should be levelled off in order to obtain an accurate reading. This can be easily done by use of the wire. Read the volume of water in the tube estimating to hundredths of a cubic centimeter and calculate the percentage of moisture by multiplying this figure by 100 and dividing by the weight of sample taken.

2. *Solubility Index.* Many operators failing to appreciate the importance of standard equipment for stirring these samples have used various types of electric mixers. Even those of the same make may vary in r.p.m. and type of agitator. We recommend the Dumore Drink Mixer No. 6 as being less expensive and more satisfactory. Fifty cc conical sediment tubes should be graduated in 0.1 divisions from 0 to 1 cc, in 0.2 divisions from 1 cc to 2 cc, in 0.5 divisions from 2 cc to 10 cc, 1 0 cc division from 10 to 20 cc, and at the 50 mark which should be at least one half inch from the top of the tube. When determinations are made on the same sample at a given laboratory results should check within the subdivision of the graduation on the sediment tube at the point where the reading is taken

4. *Flavor and Odor.* Samples of dry skim milk should be examined for odor immediately after the containers are opened and the flavor and odor should be determined on the reconstituted sample approximately 1 hour after reconstitution.

5. *Bacterial Count.* After an exhaustive study of available methods of determining bacterial count particularly with the view of correlating laboratory findings with actual quality, it was concluded that the standard nutrient agar plate method, as published in the latest edition of "Standard Methods of Milk Analysis" approved by the American Public Health Association, be adopted. In addition, the committee recommends a 10 gram

sample of the dry skim be weighed directly into a 6 or 8 ounce bottle containing 100 cc of sterile water and furnished with a sterile rubber stopper. The mixture must be very vigorously and thoroughly shaken at this point to free the solution as completely as possible of all lumps and assure homogeneous bacterial distribution. The use of lead shot size No. 7 was found advantageous in this regard. The necessary further dilutions should be made so that the colony count will not be less than 30 and not more than 300 per plate. Incubation should be for 48 hours at 37° C.

DRY SKIM MILK FOR ANIMAL FEED

Animal feeds of quality and guaranteed composition are the foundation of proper animal nutrition. The quantity of dry skim milk used for such purposes is constantly increasing. Formerly an inferior quality of product, unfit for human consumption, was acceptable; but as science has brought to light those factors that are important in proper animal nutrition, there has developed a demand for a better quality of dry skim milk for such purposes.

The following standards are to apply at the time of delivery by the manufacturer.

GENERAL REQUIREMENTS FOR CHOICE AND STANDARD GRADES

All dry skim milk for animal feed shall conform in all respects to Federal and State regulations in force at the present time or hereafter enacted respecting commercial feeds. Factories and equipment used in the manufacture of such a product shall be maintained in a clean sanitary condition. The dry skim milk shall be made from freshly skimmed milk to which no preservative, alkali, or other neutralizing agent has been added and which has been pasteurized in the liquid state either before or during manufacture at a temperature of 142° for 30 minutes or its equivalent in bacterial destruction.

It shall be reasonably uniform in composition, the color shall be white or light cream, and free from a brown or yellow color typical of overheated stock and free from any unnatural color. In dry form and in re-solution it shall be sweet and clean and free from acid, burnt, rancid, soapy, or other equally objectionable flavors or odors. A slight storage or slightly scorched flavor is permissible.

SPECIFIC REQUIREMENTS FOR CHOICE FEED GRADE

In addition to the general requirements the following are necessary:

Moisture not greater than 5.00%

Crude protein* M.F.B. (factor $N \times 6.25$) not less than 34.50%

Mineral ash* M.F.B. not more than 9.00%

Titratable Acidity* M.F.B. not more than 1.85% as lactic acid.

Sediment not greater than Disc No. 4.

* M.F.B. signifies moisture-free basis.

SPECIFIC REQUIREMENTS FOR STANDARD GRADE

Any dry skim milk failing to meet in one or more particulars the requirements of Choice Grade, shall be classed as Standard Grade providing it meets the general requirements and, in addition, the following:

Moisture not more than 6.00%
Crude protein² M.F.B. (factor $N \times 6.25$) not less than 33.00%
Mineral ash² M.F.B. not more than 9.00%
Titratable acidity² M.F.B. not more than 2.30%
Sediment not greater than Disc No. 6.

In order that laboratory results on total protein, mineral ash and titratable acidity may be made comparable in different laboratories it is recommended that they be expressed on the moisture-free basis. This is made necessary by the fact that practically all dry skim milk for animal feed is packed in bags. This product at the time of manufacture has a moisture content of 1.50 to 3.00 per cent. During transit and storage the moisture absorption increases considerably, causing wide variations in the percentage of other ingredients when determined on the wet basis.

The factor 6.38 should be used for calculating milk protein where exact results are desired. Feed manufacturers customarily use the factor 6.25 for calculating vegetable proteins in animal feed and prefer to use this factor for total protein of all feed mixtures. It is, for this reason, the committee has suggested the use of this factor for milk protein but only in cases where it particularly applies to animal feed and serves as a basis for calculating total calorific value.

SAMPLE FEED GRADE

Any dry skim milk failing in one or more particulars to meet the requirements of Standard Feed Grade shall be classed as Sample Feed Grade.

METHODS OF SAMPLING AND ANALYSIS

The methods of sampling and analysis are the same as those outlined in this JOURNAL, Volume XIII, No. 4, July 1930, for examination of dry skim milk for human consumption. In addition, the following are recommended:

Crude Protein. This should be determined by the method outlined for the determination of protein in grain and stock feeds. See the Journal of the Association of Official Agricultural Chemists.

Mineral Ash. Weigh accurately a 1 gram sample into a platinum or porcelain dish, ignite in a muffle furnace at 600° C. to 700° C. (dull red heat). It is important to increase the heat very gradually and continue the heat until the residue is free from carbon and shows a light gray or white appearance.

A PLAN AND PRELIMINARY RESULTS OF A PERMANENT PASTURE GRAZING TEST*

E. C. ELTING AND J. P. LAMASTER

South Carolina Agricultural Experiment Station, Clemson College

During the growing seasons of 1929 and 1932 inclusive, 46 plots of old established bermuda pasture sod were used to determine the effect of various fertilizer treatments on the yield and composition of this pasture grass.

The plots were each one two-hundredth of an acre net in size with suitable borders. They were clipped at regular two-week intervals throughout the growing season, green yields determined, and samples taken for chemical analysis.

The soil of these plots is of the Cecil sandy clay loam type with an average pH of 5.3.

Fifteen different fertilizer combinations were used on both a limed and an unlimed series, all applications being made at the rate of 600 pounds per acre for each designated formula.

At the beginning of the test the limed series received an application of ground dolomitic limestone at the rate of two tons per acre.

The results of this plot work show that the only profitable fertilizer treatment for pasture on this soil type is lime and phosphorus.

The application of lime alone increased the yield 33.6 per cent over the untreated plots, phosphorus alone showed an increase over the untreated of 41 per cent, lime and phosphorus showed an increase of 79.5 per cent over the untreated, 34.3 per cent over the lime alone, and 27.2 per cent over the phosphorus alone.

During 1933 a grazing test was conducted to determine the value of established bermuda pasture under three systems of fertilization.

SIGNIFICANT FEATURES OF PASTURE GRAZING PLAN

The general plan of this test is based on the assumption that fixed charges in the cost of milk production other than the cost of feed, remain approximately the same regardless of season, increased refrigeration costs in summer tending to offset cost of bedding, handling manure, and increased labor of feeding in winter.

In order to get profitable production from cows throughout their lactation period it is essential that the level of production be held up sufficiently during pasture season so that the cows are still producing at a profitable level when they go into winter feeding conditions.

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* Technical contribution number 31 of the South Carolina Experiment Station, Clemson College, S. C.

The most significant features embodied in this grazing plan are:

1. Using comparatively small pasture units with carefully controlled conditions.
2. Stocking pasture at a minimum rate of one cow per acre, adding or removing animals above one cow per acre as conditions of pasture seemed to justify.
3. Using the same cows throughout the entire grazing period so far as possible, cows added to be handled under similar conditions prior to being placed on test. Using only cows which have been in milk at least six weeks.
4. Weighing animals weekly, keeping daily milk yields, and testing weekly composite milk samples for butterfat.
5. Barn feeding at such rate as to maintain the milk yield above a maximum decline rate of 2.5 per cent per week.
6. Keeping live weight variations at a maximum of 10 per cent of the initial weight for the season.
7. Providing movable cages, three per pasture, each 1/1500 acre in size in order to obtain clipped samples for yield and composition determinations. Caged areas clipped at two-week intervals and the cages moved to a new location.
8. Expressing pasture returns in terms of T.D.N. (total digestible nutrients) produced. This figure is based on the total requirement of cows on pasture for maintenance, milk production, and live weight change less the amount of T.D.N. supplied in barn feeding. These results may be readily converted to their equivalent in alfalfa hay or other types of feeds.

DESCRIPTION OF PASTURES AND TREATMENT

Three two acre pastures of established bermuda sod were used in this test.

Lots 1 and 2 had, previous to 1930, been in cultivation. These lots were sodded with bermuda in 1930. Lot 3 was an old established sod formerly a part of the regular dairy herd pasture.

Lots 1 and 2 received an application of 3000 pounds of dolomitic limestone per acre in the spring of 1931 and were continuously grazed during the season of 1932.

In March, 1933, Lot 1 received an application of 450 pounds of 16 per cent superphosphate per acre. Lot 2 received no further treatment. Lot 3 received an application of 3000 pounds of dolomitic limestone per acre in the fall of 1932. This lot had a considerable accumulation of manure, which was evenly distributed over the area early in 1933, and which constituted the only fertilizer treatment for this lot.

Lots 1 and 2 had a perfect stand of hop clover (*Trifolium procumbens*) ready for grazing on April 5, 1933.

This plant furnished practically 100 per cent of the grazing on these

pastures up to May 17, after which date bermuda (*Cynodon dactylon*) gradually replaced it and continued to be the only available grass for the remainder of the season.

Lot 3 contained only a small amount of hop clover (estimated at 10 per cent) and did not furnish grazing until April 19, with bermuda furnishing the bulk of the grazing throughout the season.

FEEDING AND MANAGEMENT OF COWS

All cows selected for the start of this trial were in rather heavy production, at least six weeks advanced in lactation, with one exception, and yet early enough in lactation so that they could be expected to be used throughout the season. All cows were milked three times per day throughout the trial.

At no time was barn feeding entirely discontinued. A grain mixture containing 14.4 per cent digestible protein and 71.3 per cent T.D.N. was fed up to July 26, and another grain mixture containing 15.4 per cent digestible protein and 74.1 per cent T.D.N. was fed during the remainder of the trial.

Grain was the only feed allowed in the barn except during the last two weeks of the trial (Oct. 11-25) when corn silage was added to the ration.

The cows had access to a salt block and drinking cups at all times, and shade was provided.

RESULTS

Table 1 shows the rate at which the pastures were stocked throughout the season by weekly periods, the average yield per cow of fat corrected four per cent milk produced by weeks along with the calculated average base yield computed on the basis of a maximum decline of 2.5 per cent per week. Each week's base production equals 97.5 per cent of the base yield of the preceding week. The calculated base was corrected each time a change of cows, either in number or individuals, was made.

The average weight for each cow used in this trial was calculated by totaling the weekly weights and dividing by the number of weeks she was on pasture. The average weight of each cow was multiplied by the number of days she was on pasture and the total of these weights was divided by one thousand to get the number of pasture days for a thousand pound cow.

The total increase in weight for cows in each pasture represents the total of the differences between initial and final weight for each cow.

The actual yield of milk and butterfat produced by each group was converted to a four per cent milk basis by the formula of Gaines and Davidson,¹ (4 times the milk plus 15 times the butterfat).

¹ Gaines, W. L., and Davidson, F. A. Relation between percentage fat content and yield of milk. Ill. Experi. Sta. Bulletin 245, 1923.

TABLE 1
The rate of stocking and comparison of actual and calculated base yield of fat corrected 4 per cent milk by weeks

DATE WEEK ENDING	LOT 1			LOT 2			LOT 3		
	Cows per acre	Milk per cow week		Cows per acre	Milk per cow week		Cows per acre	Milk per cow week	
		Actual avg	Calculated base avg.		Actual avg.	Calculated base avg.		Actual avg	Calculated base avg.
		lbs.	lbs.		lbs.	lbs.		lbs.	lbs.
4-12	1.0	359.2	359.2	1.0	318.0	318.0			
4-19	2.0	334.8	304.8	1.0	321.0	310.0			
4-26	3.0	305.7	278.8	1.5	308.6	287.8	1.0	275.9	275.9
5-3	3.0	300.4	271.8	1.5	304.7	280.6	1.0	301.4	269.0
5-10	2.0	291.3	282.5	1.5	283.6	273.6	1.5	258.7	267.2
5-17	2.0	276.9	275.4	1.5	283.0	266.7	2.0	246.5	250.2
5-24	1.0	296.2	308.5	1.0	260.5	273.0	2.0	231.2	243.9
5-31	1.0	269.7	300.7	1.0	246.4	266.2	2.0	240.9	262.6
6-7	1.0	284.8	295.7	1.0	253.2	259.5	1.5	268.1	257.7
6-14	1.0	270.5	285.8	1.0	236.0	253.1	1.5	252.2	251.2
6-21	1.0	267.1	278.7	1.0	234.3	246.7	1.5	241.0	244.9
6-28	1.0	257.8	271.7	1.0	230.7	240.6	1.0	251.1	262.1
7-5	1.0	277.2	264.9	1.0	244.3	234.5	1.0	271.2	255.5
7-12	1.0	291.4	258.3	1.0	244.7	238.6	1.0	285.1	249.1
7-19	1.5	228.1	216.5	1.5	200.7	198.1	1.5	246.7	220.6
7-26	1.5	217.1	211.1	1.5	200.2	193.1	1.5	230.3	215.1
8-2	1.5	220.7	205.7	1.5	202.4	188.3	1.5	233.1	209.7
8-9	2.0	207.6	204.8	1.5	191.4	183.6	2.0	225.6	217.9
8-16	2.0	201.5	199.7	1.5	186.5	179.0	2.5	214.6	202.7
8-23	2.5	205.5	210.2	2.0	196.2	192.7	2.5	209.3	205.5
8-30	2.5	193.1	205.1	2.0	179.8	187.9	2.5	196.5	200.3
9-6	2.5	182.8	199.9	2.0	200.9	201.2	2.5	190.4	195.3
9-13	2.5	176.1	194.9	2.0	191.0	196.2	2.5	176.6	190.4
9-20	2.5	167.9	190.0	2.0	187.0	191.3	2.5	194.5	194.4
9-27	2.0	178.6	205.8	2.0	177.1	186.4	2.5	189.2	189.5
10-4	2.0	167.8	200.6	2.0	168.7	181.9	2.5	166.0	184.7
10-11	2.0	160.0	195.6	2.0	161.5	177.2	2.5	149.0	180.1
10-18	2.0	164.8	190.7	2.0	157.1	172.7	2.5	154.2	175.6
10-25	2.0	156.2	185.9	2.0	147.3	168.4	2.5	140.1	171.3

The total requirements for digestible nutrients for each group are expressed as a single figure.

The requirements for maintenance and milk production were calculated by the Morrison Standard² using the minimum figure in the requirement for milk yield.

The requirement for gain in weight was calculated the basis of Armsby's³ data which shows that 3.25 therms of net energy are required on the average for one pound of increase in weight, and on the assumption that one pound of total digestible nutrients is approximately equal to a therm of net energy.

A summary of the total carrying capacity of the pastures, the total milk produced, the total nutrient requirement, and the net amount of nutrients obtained from pastures are presented in table 2.

TABLE 2
Summary of pasture returns per acre, season of 1933

	LOT 1	LOT 2	LOT 3
Calendar days continuous grazing	203.0	203.0	189.0
Cow days (1000 lbs. avg. wt.)	368.0	304.5	377.9
Total gain in wt. lbs.	86.5	102.5	110.0
Total 4 per cent milk lbs.	12118.7	9369.6	11055.6
T.D.N. requirement	6971.3	5633.7	6782.9
T.D.N. fed in barn	2349.3	1939.2	2087.9
T.D.N. from pasture	4622.0	3694.5	4695.0
Alfalfa hay equivalent from pasture, tons	4.478	3.580	4.549
Alfalfa hay equivalent per day per cow from pasture, lbs.	24.3	23.5	24.0
Percentage of T.D.N. requirements for maintenance from pasture	158.5	153.1	156.8
Percentage of T.D.N. required furnished by barn feeding	33.7	34.4	30.8

From the clipped samples obtained from the caged areas, green yield determinations and complete feed analysis were made by two-week intervals throughout the grazing season.

The green yield per acre, the percentage of dry matter in the green grass, and the average composition of the dry matter along with the total rainfall for each two week-period are presented in figure 1.

DISCUSSION OF RESULTS

Figure 1 shows the extreme variations in green yield, dry matter yields, and nutrient content of pasture grass through the growing season. In order to meet the changing values of these pastures it is necessary to vary

² Feeds and Feeding, Henry and Morrison, 18th Edition, 1923, Henry and Morrison Co., Madison, Wis., Appendix Table V.

³ The Nutrition of Farm Animals, Armsby, 1922, McMillin Co., New York, Appendix Table III.

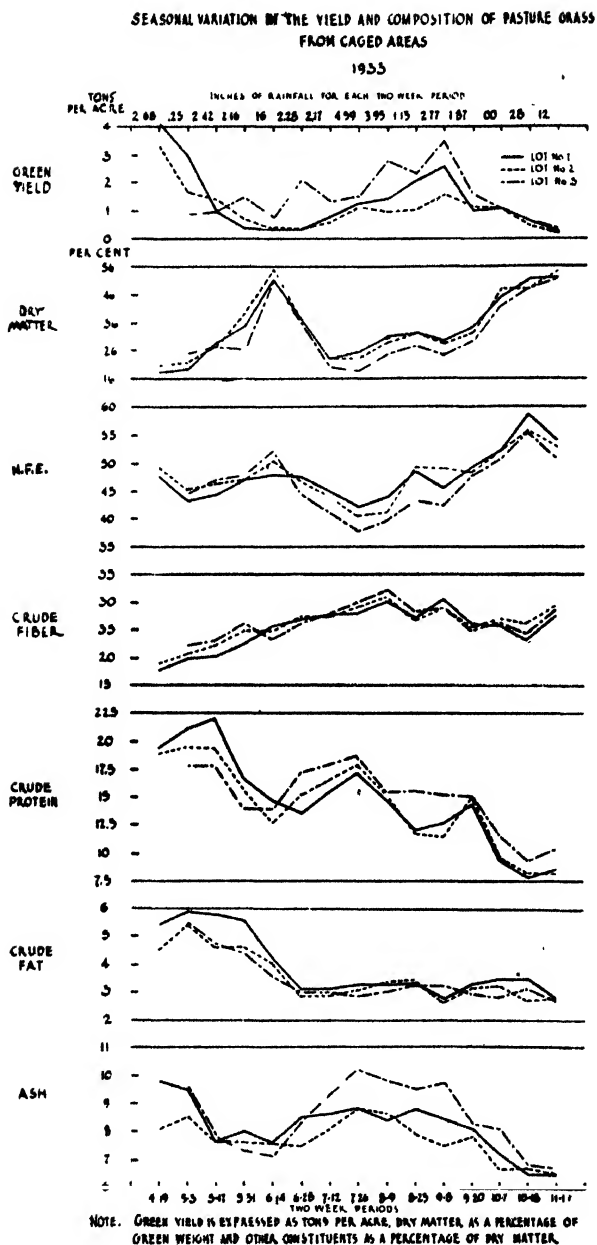


FIG. 1

both the rate of stocking and the level of barn feeding. If these variations are not made to meet these changing conditions, the cows will decline in milk production beyond reasonable limits during periods of slow growth, which result largely from deficient rainfall.

With this in mind, it is our plan to keep the pastures stocked at a minimum of one cow per acre, adding cows as the conditions of the pasture seem to justify and feeding in the barn at such rate as to maintain a level of production at or above a maximum decline of 2.5 per cent per week. Since all cows, with one exception, used in this work were at least six weeks advanced in lactation and had reached their normal maximum production, we arbitrarily set a decline rate of 2.5 per cent per week as a rate that would permit satisfactory production throughout their lactation period.

Table 1 shows how nearly these conditions were met under actual practice both as regards varying the pasture load and maintaining the expected level of production.

The greatest deviation from the base milk yields as described above occurred in June and during the last few weeks of the growing season when extremely dry weather prevailed. Also in order to get all available grazing by the end of the season the pastures were heavily stocked during the last few weeks of the trial.

In considering the data in table 2 attention is called to the large amount of milk produced per acre under this plan of management.

In measuring the value of the various fertilizer treatments on these pastures, using the pounds of T.D.N. secured as the returns, it is noted that Lot 1 which received 450 pounds of 16 per cent superphosphate per acre yielded 927.5 more pounds of T.D.N. per acre than Lot 2 which was treated in the same way in every respect except for the application of phosphate fertilizer. This amount is equivalent to 1796 pounds of alfalfa hay per acre directly attributable to the application of phosphate fertilizer. Without giving credit for any residue that may be carried over into following seasons this fertilizer application represents a total cost of \$3.55 per acre (\$3.15 for superphosphate plus 40 cents labor for applying). Lot 3, which received a heavy application of manure as the only treatment, yielded 1000.5 more pounds of T.D.N. per acre than Lot 2, or an alfalfa hay equivalent of 1938 pounds per acre. Since this manure applied to Lot 3 represents an accumulation rather than a direct application, no definite cost can be assigned to it.

As an observation it is noted that this heavy application of manure had no detrimental effect on the palatability of the grass as indicated by the completeness of grazing of all available growth on this lot.

The high alfalfa hay equivalent secured from all these pastures as shown in table 2 indicates that bermuda grass may be ranked as a very satisfactory plant for dairy pastures. When supplemented with hop clover, at least two weeks earlier grazing may be secured.

IRRADIATED MILK: THE TRANSMISSION AND ANTIRACHITIC ACTIVATION OF MILK FILMS BY ULTRA- VIOLET RADIATIONS

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Data previously reported have shown the degree of antirachitic activity imparted to milk by different sources and intensities of ultraviolet radiation (1, 2, 3). It was found that a low intensity of radiation applied for relatively long periods of time was not as effective in increasing the antirachitic potency as radiations of high intensity applied for shorter periods of time, the total energy applied per unit amount of milk being substantially the same in both instances. These observations were obtained under controlled commercial conditions involving large quantities of milk irradiated as flowing films about 0.4 millimeters thick and for time periods varying from 8 to 48 seconds. While this procedure is not considered to have affected the validity of the conclusions, nevertheless, it seemed desirable to undertake more detailed studies designed to determine the degree of activation as affected by such interrelated factors as, depth of penetration of the activating rays, characteristics of the irradiated film and time of exposure. The previous data were obtained by irradiating milk films flowing over corrugations on a vertical supporting surface; such corrugations interfere with the normal interplay of frictional, gravitational and surface tension forces which are operative in determining the character of flowing films. For the present work a smooth, flat, vertical surface was used for producing flowing films of known thickness and velocity.

EXPERIMENTAL

Milk films of varied and known thickness were formed on a smooth vertical surface 40×56 cm. in area. The milk was allowed to flow from a horizontal slot which perforated the face of the supporting surface at the top. The width of the slot was such that the surface tension of the milk permitted complete closure of the slot irrespective of the rate of flow. Flowing films of uniform thickness and character could be formed over the area as desired.

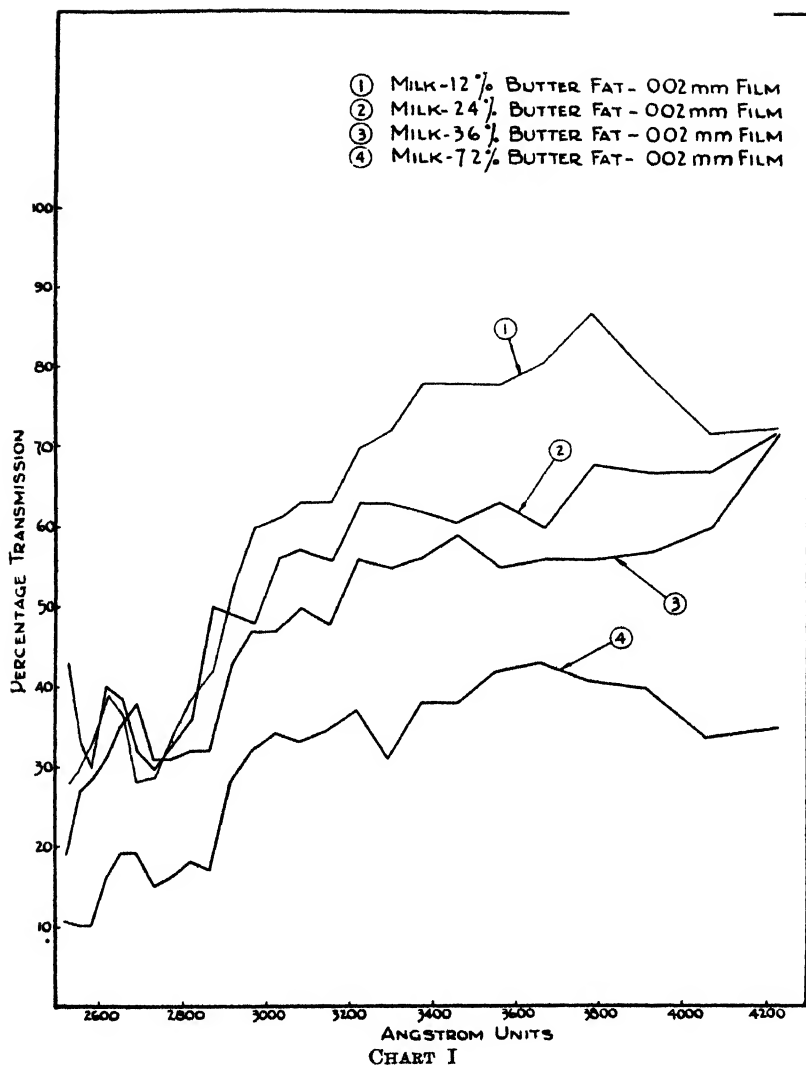
The thickness of the various milk films was determined by suitable calculations involving the quantity of milk delivered by a given area of film per unit of time, and the time required for the film to travel a given vertical

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distance. The rate of speed of the flowing films was determined by placing on the surface of the film small amounts of an appropriate material (special grade of finely divided graphite), and with the aid of a high speed motion-picture camera determining the time, in fractions of a second, required for such material to travel a given distance. The result thus obtained is considered to be the average speed of flow in contradistinction to the speed of the surface, or the speed at the interface between the milk and the board.

The depth to which the activating ultraviolet rays penetrated the milk was determined by measuring the percentage transmission through films or layers of known thickness. A carbon arc lamp, burning C type carbon electrodes at 60 amperes and 50 volts, was used. Radiations from this arc produce an enormous number of lines so close to each other that an approximately continuous spectrum is obtained throughout the ultraviolet, thus permitting spectral transmission measurements in all portions of this region. The lamp was an automatically controlled, motor fed unit giving a uniform and constant intensity of radiation (1). Vertical flowing milk films were formed over the flow board into which had been inserted a smooth quartz plate 5 cm. square, whose surface was in the same plane as the surface of the board. The films flowed over the quartz plate uniformly without distortion or disturbance. The flow board with the quartz window was placed at right angles to the beam of radiation 43 cm. from the arc. The percentage radiation of various wave lengths transmitted by the milk films was determined by comparing the intensity of the radiation of particular parts of the spectrum with and without the milk in the path of the beam. Measurements were made with a spectroradiometer placed back of the milk film at such a distance that the first lense was 63.5 cm. from the film. A piece of quartz plate with a "ground glass" or diffusing surface was placed behind the board in the path of the transmitted beam to act as a secondary radiation source and thus reduce the errors due to scattering of the radiation transmitted by the film.

The spectroradiometer was the instrument used in the previous work (1, 4, 5, 6). A quartz prism dispersed the radiation into a spectrum. A sodium photo-electric cell was moved from point to point through the spectrum, thus actuating the measuring galvanometer. The intensity of radiation of successive small bands of progressively different wave lengths was determined. The procedure was to obtain the readings of the galvanometer for each of the stations throughout the spectrum when the lamp was burning, but with no milk film present. The milk film was then established and the measurements were again taken. Usually another blank run was made again after the milk film was discontinued in order to check on the constancy of the radiation source and apparatus. Transmission data from a variety of milk derivatives, as well as from milk of variable fat content, were obtained for films 0.02, 0.11 and 0.23 millimeters thick. The



The transmission of ultraviolet radiations by milks of variable fat content (films 0.02 mm. thick).

transmission through layers 6 millimeters thick were obtained for certain substances by using a quartz cell. The curves shown herein (Charts I, II, III and IV) represent the average results of from three to five determinations.

Milk of varying fat content was prepared by mixing suitable proportions of skim milk or cream with natural whole milk containing 3.6 per cent butter fat.

The whey was similar to that obtained in the manufacture of cheese,

wherein rennet extract and slight acidification (pH 6.1) of the milk was used for precipitating the casein.

Albumin-free whey was obtained by adjusting the pH of the whey to 4.55 with dilute hydrochloric acid and heating to the boiling-point; the coagulated albumin was removed by filtration. Such treatment, however, does not remove the total proteinaceous matter.

The product designated as the water soluble milk vitamin concentrate represents a concentration of those residual substances obtained from milk

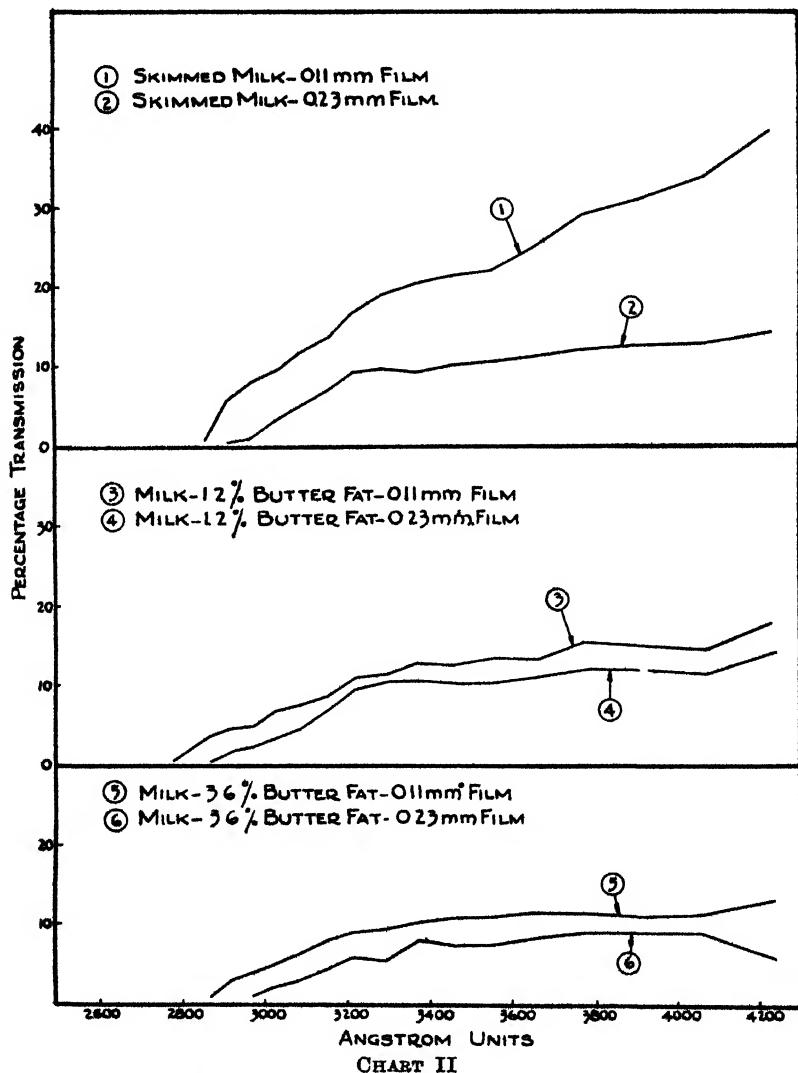


CHART II
The transmission of ultraviolet radiations by milks of variable fat content (films 0.11 and 0.23 mm. thick).

after removal of the fat, casein, albumin, most of the calcium and inorganic phosphorus, and a very large proportion of the original lactose (7).

The milk derivative designated as protein sample No. 31 was obtained by subjecting a freshly precipitated casein suspension to a series of washings in an aqueous medium with adjusted pH value and a small amount of an ionizing salt. This product has marked surface tension reducing properties; it contains a lower amount of nitrogen than the commonly known milk

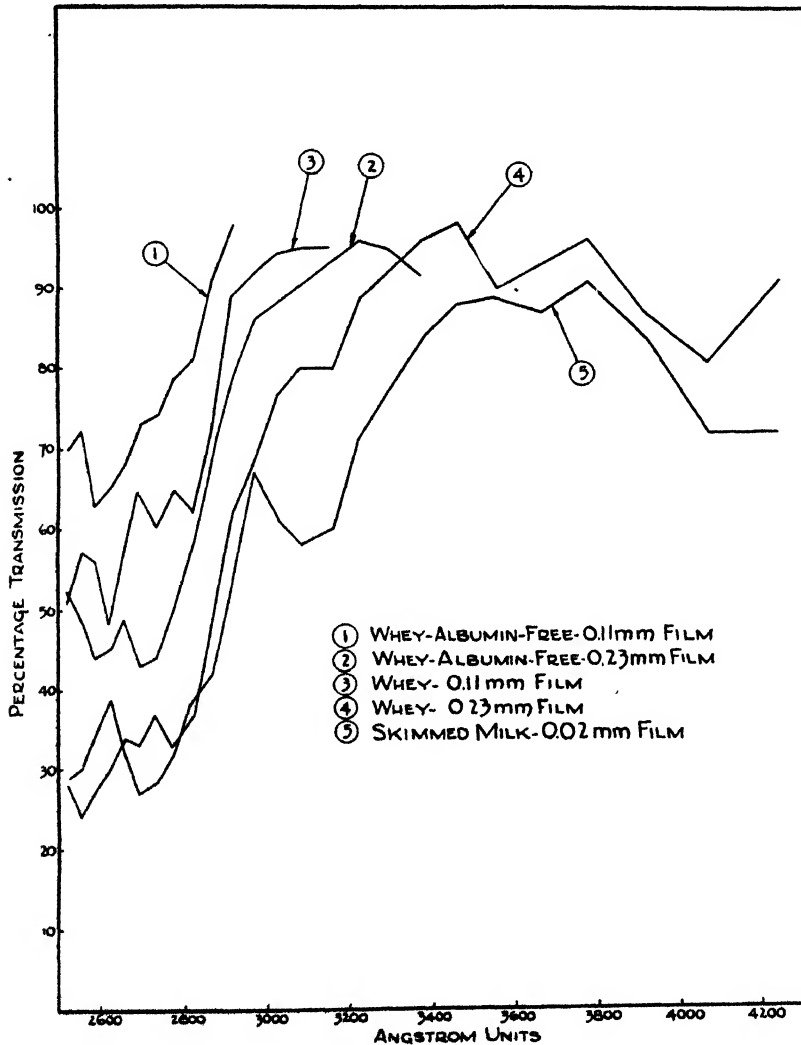


CHART III

The transmission of ultraviolet radiations by skimmed milk and whey through films of variable thickness.

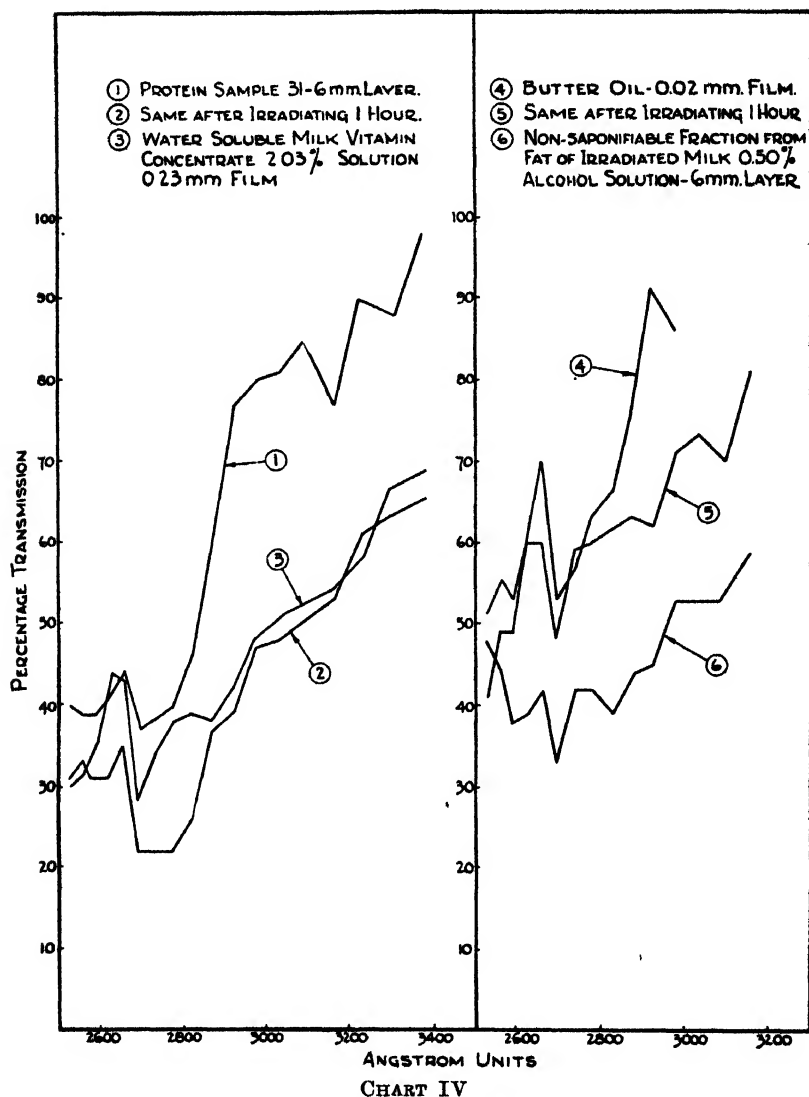


CHART IV

The transmission of ultraviolet radiations by different milk substances through films of variable thickness.

proteins and sulphur, but no cystine. It has certain characteristics similar to those reported for lactoglobulin.¹ The ultraviolet transmitting properties were obtained for a clear solution containing 0.04 per cent of this substance.

The butter oil was obtained in the usual manner by filtering melted butter fat.

¹ Other characteristics of this substance are under investigation.

The non-saponifiable fraction of the butter fat was obtained by the usual method of saponification with alcoholic potassium hydroxide, the fat having been extracted by alcohol and ether from dry milk irradiated prior to desiccation.

Since one of the objects of this work was to correlate the physical data with the antirachitic activity imparted to various milks and milk derivatives under controlled conditions of irradiation, certain of the products examined radiometrically were subjected to the usual Vitamin D assay (2) after irradiating under the same conditions as were used for obtaining the transmission data. It was desirable, and in some instances necessary, to use arbitrary periods of exposure. Such periods could be readily determined and controlled for the various films by utilizing the information regarding speed of flow and capacity obtained as previously described. The biological and physical data are recorded in Table I. The method of procedure and calculations formerly employed were used (1, 2, 3). The energy calculations are for the 2000–3000 \AA range. The amount of irradiated substances required to give a 1+ line test (8, 9) was the criterion for determining a uniform and consistent antirachitic effect. The test substances were fed for a 10-day period following a 21-day period on the Steenbock rachitogenic diet No. 2965 (10).

DISCUSSION

The transmission data show that extremely thin films of milk and many milk derivatives are relatively opaque to the antirachitic ultraviolet rays; this is especially true for those wave lengths below about 2850 \AA . It appears that from 60 to 80 per cent of the incident radiation below about 2850 \AA is absorbed by the first 0.02 millimeter depth; 95 per cent or more is absorbed by the first 0.11 millimeter depth. The depth of penetration is decreased as the fat content of the milk increases; however, this decrease in depth of penetration does not follow the fat content proportionately. The percentage transmission between about 2500 and 2850 \AA by films 0.02 millimeters thick is substantially the same for whole milk containing 3.6 per cent fat, milk containing 1.2 per cent fat and skimmed milk. While the fat content of the milk is indicated as affecting the opacity of milk to ultraviolet rays it is obvious that substances other than fat are largely responsible for the screening effect observed.

The data from the milk derivatives show that each of the substances examined absorb substantial proportions of the incident energy within the antirachitic range. Butter oil in films of 0.02 millimeters thickness transmit the ultraviolet radiations to about the same degree as milk films of similar thickness. The transmitting properties of whey and albumin-free whey are substantially the same for films 0.23 millimeters thick as the 0.02 millimeter films of milk with low fat content. This relationship was not

TABLE I
The vitamin D concentration of irradiated milk as affected by film thickness, fat content and amount of applied energy
 (2000-3000 Å°)

SAMPLE	TEST SUBSTANCE	FILM THICKNESS	EXPOSURE PERIOD	TOTAL ERGS PER CC. (X10 ⁶)	TOTAL QUANTA PER CC. (X10 ¹⁶)	TOTAL MILK FED	VITAMIN D PER CC. (X10 ¹⁰)	VITAMIN D PER MG. OF FAT (X10 ¹⁰)
P31-2	Milk protein No. 31	mm. 6.00	secs. 90.0			cc 160*	mola. Negative	mola.
2XXX-6	Milk vitamin concentrate							
RW-6	Whey	0.02	48.0	429,360	538,830	203*	45	
8-1	Skimmed milk	0.02	16.0	143,120	179,610	60	75	
0G-1	Milk, 0.6% fat	0.02	16.0	143,120	179,610	50	90	299.70
1A1	Milk, 1.2% fat	0.02	1.0	8,945	11,851	60	75	12.50
3A1	Milk, 1.2% fat	0.02	1.0	8,945	11,851	60	75	6.25
6A1	Milk, 1.2% fat	0.11	1.87	3,769	4,994	20	225	18.75
1AW1	Milk, 3.6% fat	0.23	0.89	972	1,288	50	90	7.50
3AW2	Milk, 3.6% fat	0.02	1.0	8,945	11,851	30	150	4.16
6AW2	Milk, 3.6% fat	0.11	1.87	3,769	4,994	20	225	6.25
1AW7	Milk, 7.2% fat	0.23	0.89	972	1,288	35	128	3.55
3AW7	Milk, 7.2% fat	0.02	1.0	8,945	11,851	17.5	257	3.56
B0-6-48	Filtered butter oil	0.11	1.87	3,769	4,994	17.5*	257	3.56
		0.02	1.0	8,945	11,851	300-600**		15.00-7.5

* Milligrams dry substance.

** Results from different samples of butter oil have been quite variable.

unexpected; the results show that the fat and casein of milk are largely responsible for the opacity of milk to ultraviolet rays. The 0.04 per cent solution of protein sample No. 31 examined in a 6 millimeter layer, while showing relatively greater transmitting properties than the other products, nevertheless absorbs ultraviolet rays within the antirachitic range to a substantial degree.

The data as a whole show that the opacity of natural milk to ultraviolet radiations is the result of the inherent combination of many individual milk constituents of widely different character. The similarity of the transmission curves within the antirachitic range is greater than in the longer ultraviolet region. The specific and similar character of the curves can be interpreted as possible evidence of the presence of a specific molecular entity common to each of the milk derivatives. However, such conclusions cannot be accepted with finality at this time.

The biological data show that many of the products are capable of being antirachitically activated to a greater or less degree under the conditions used. These results interpreted in conjunction with the transmission data, and the short periods of time required to produce a substantial degree of activation, leads to the conclusion that Vitamin D synthesis in milk takes place at, or substantially at, the surface of the milk and that the character of the milk film is an important feature determining the degree of activation obtained under given conditions of treatment. It is apparent that with the high intensity of radiations used in these experiments the thinnest attainable films were not optimum for the most efficient and effective activation. The relative activability of the milk derivatives and the milks containing variable amounts of fat show that with these conditions of irradiation involving only momentary periods of exposure, there is only an apparent relationship between the fat content of the milk and the antirachitic effect obtained. It is obvious that the degree of activation does not parallel the fat content.

It has been shown in a previous paper (3) that a high intensity of radiation applied for short periods is more effective in activating milk films 0.4 millimeters thick than a low intensity applied for longer periods of time, the energy applied per unit amount of milk being substantially the same in both cases. The data presented in the present paper show that the opacity of the milk greatly reduces the intensity of the incident radiations at very slight depths below the immediate surface. It appears, therefore, that a low intensity of radiation will proportionately reduce the effectiveness of the energy available for activation of the pro-vitamin at any depth of the milk layer below the immediate surface. The evidence shows that suitable flowing films which constantly bring fresh material to the surface permit a greater degree of activation under given conditions than the slowly

moving, extremely thin films. The results of further studies concerning this aspect of milk irradiation will be presented in a subsequent paper.

CONCLUSIONS

1. Only 20 to 40 per cent of the ultraviolet radiations between 2500 and 2850 \AA striking the surface of the milk at right angles are transmitted by films 0.02 millimeters thick. Milk films 0.11 millimeters thick transmit 5 per cent or less of the incident radiation between these limits.

2. As the fat content of the milk is increased the ultraviolet transmitting properties are decreased somewhat but not in proportion to the amount of fat present.

3. The transmission curves of certain milk derivatives of widely different character are similar to those of the natural whole milk and milks containing variable amounts of fat.

4. Milks containing variable amounts of fat and certain milk derivatives are more transparent to long wave ultraviolet radiations than to the short wave radiations. This increase in transmission occurs rapidly and progressively in the region 2800–3300 \AA .

5. The degree of antirachitic activation imparted to milk and milk derivatives bears only an apparent general relationship to the fat content; the antirachitic properties obtained under the conditions of treatment used are not in proportion to the amount of fat present. Relatively high antirachitic properties are shown by the irradiated milks containing low amounts of fat.

6. The opacity of milk necessitates the utilization of relatively high intensities of ultraviolet radiation for efficient and effective activation. Under suitable conditions a high degree of activation can be obtained by an exposure period varying from less than 1 second to not more than 2 seconds.

7. The effectiveness and efficiency of utilization of the ultraviolet radiations used for activating milk is influenced by the character and thickness of the milk film; extremely thin, slow moving films showed a less degree of activation, after a momentary exposure, than did thicker and faster moving films.

8. It is probable that the synthesis of Vitamin D by direct irradiation takes place at, or substantially at, the surface of the milk.

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REFERENCES

- (1) SUPPLEE, G. C., DORCAS, M. J., AND HESS, A. F. Irradiated Milk: The Energy Requirements for Antirachitic Activation. *Jour. Biol. Chem.* **94**: 749. 1932.

- (2) SUPPLEE, G. C., HANFORD, Z. M., DORCAS, M. J., AND BECK, H. H. Irradiated Milk: The Amount of Vitamin D and its Rate of Formation. *Jour. Biol. Chem.* **95**: 687. 1932.
- (3) SUPPLEE, G. C., BECK, H. H., AND DORCAS, M. J. Irradiated Milk: The Influence of the Intensity and Character of the Radiations on the Antirachitic Potency. *Jour. Biol. Chem.* **98**: 769. 1932.
- (4) DORCAS, M. J. Ultra-Violet Radiations in Industry. *Ind. and Eng. Chem.* **22**: 1244. 1930.
- (5) GREIDER, G. E. Energy Emission Data of Light Sources for Photochemical Reactions. *Ind. and Eng. Chem.* **23**: 508. 1931.
- (6) COBLENTZ, W. W., DORCAS, M. J., AND HUGHES, C. W. Radiometric Measurements on the Carbon Arc and other Light Sources used in Photo Therapy. Bureau of Standards, Sci. Paper 539. 1926.
- (7) SUPPLEE, G. C., DOW, O. D., AND FLANIGAN, G. E. The Concentrated Water Soluble Fraction of Milk as a Source of Vitamin B. *JOURNAL DAIRY SCIENCE* **11**: 420. 1928.
- (8) BILLS, C. E., AND McDONALD, F. G. Antirachitic Substances: IV. The Polymerization of Cholesterol. *Jour. Biol. Chem.* **68**: 821. 1926.
- (9) BILLS, C. E., HONEYWELL, E. M., WIRICK, A. M., AND NUSSMEIER, M. A Critique of the Line Test for Vitamin D. *Jour. Biol. Chem.* **90**: 619. 1931.
- (10) STEENBOCK, H., AND BLACK, A. Fat Soluble Vitamins: XXIII. The Induction of Growth-promoting Properties in Fats and their Unsaponifiable Constituents by Exposure to Light. *Jour. Biol. Chem.* **64**: 263. 1925.

THE RELATIVE VALUE OF IRRADIATED YEAST AND IRRADIATED ERGOSTEROL IN THE PRODUCTION OF VITAMIN D MILK

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In 1924 Luce (1) demonstrated that the diet of the cows was the principal factor which influenced the antirachitic value of the milk. This observation was soon after confirmed by Golding, Soames and Zilva (2) who showed further that high doses of cod-liver oil reduced the percentage of fat in the milk. Later studies by Hart, Steenbock, Teut, and Humphrey (3) indicated that the vitamin D of cod-liver oil was poorly, if at all, absorbed from the intestinal tract of the cow.

The availability of substances made highly antirachitic by exposure to ultra-violet rays has made possible a further attack of the problem of increasing the antirachitic potency of cow's milk. Thus Wachtel (4) and Steenbock and associates (5) have shown that the feeding of irradiated yeast caused a definite increase in antirachitic potency and a similar response was noted by Krauss, Bethke and Monroe (6) when irradiated ergosterol was employed.

In 1931 Thomas and MacLeod (7) in a preliminary report of a comparison of the efficiency of the two irradiated products, yeast and ergosterol, showed that 1.5 to 3 times as much irradiated ergosterol was necessary for the production of a milk of the same potency as that obtained when irradiated yeast was used. This investigation was continued and in a later report by Hess, Lewis, MacLeod and Thomas (8) it was concluded that irradiated yeast was 3 times as effective as irradiated ergosterol in the production of vitamin D milk. This difference in the efficiency of the two sources of the factor is quite striking when it is considered that isolated ergosterol is activated in one case and presumably it is the same substance which is activated in the yeast. A critical examination of the paper by

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Hess, Lewis, MacLeod and Thomas (8) revealed that the line test ratings used in estimating the potency of the butterfats were 3+ and 4+ grade. It has been our experience, as well as that of Bills and associates (9) and Dyer (10), that a considerable difference in supplement levels is required to cause a difference in rat responses of the 3+ and 4+ grade, but that when supplements are fed which produce a narrow continuous line, 1+ grade, the animal response is more sensitive to smaller differences in amounts of supplement and a more quantitative estimate of potency is possible. Hence it was considered that the type of response used by Hess and associates did not allow as quantitative an assay as was desirable and it was deemed advisable to determine again the relative efficiency of the two products in the production of vitamin D milk at various levels of feeding. Furthermore, such a study might reveal facts which would lead to a better understanding of the nature of the actions of these substances and, if a marked difference in efficiency actually existed, to methods of increasing the efficiency of the irradiated ergosterol. It was recognized that the factor developed in the yeast cell might be protected in some manner, possibly against oxidation, and therefore the use of hydroquinone, as an antioxidant, in some of the solutions of irradiated ergosterol was suggested. Consequently a series of experiments was conducted in which accurately assayed irradiated yeast and solutions of irradiated ergosterol, with and without hydroquinone, were fed to cows at various intervals, and the butterfat assayed for antirachitic potency.

THE DETERMINATION OF ANTIRACHITIC POTENCY

Young rats 24 to 28 days of age and weighing 55 to 60 grams were depleted of their stores of the factor on the Steenbock 2965 rachitogenic diet in 23 or 24 days. The amount of material to be tested, mixed with 50 grams of the rachitogenic diet, was consumed during the first 6 or 7 days of the test period. During the remainder of the 10 day test period the unsupplemented diet was fed. When it was not feasible to mix the supplement with the diet, the amount to be tested was fed separately in daily portions, during the first 6 or 7 days of the test period.

The degree of calcification was observed in the split radius and ulna essentially according to the Shipley technique (11). The following signs were used in expressing the extent of calcification:

No calcification in the metaphysis	-
Calcification just beginning	-(±)
Narrow broken line of calcification	±
Better than ± but not +	±(+)
Narrow continuous line of calcification	+
Better than + but not ++	++(+)
Medium line of calcification	++

Wide line of calcification or narrow epiphyseal cartilage	+++
Very narrow epiphyseal cartilage or complete healing	++++

When the responses of + grade, including one-half of those of \pm (+) grade, constituted 60 per cent of the total number of responses one rat unit of the antirachitic factor was said to be present in the total amount of material fed. In order to determine accurately the unit amount, an attempt was made to feed a level to which the response was slightly less than that considered to be the unit amount and one to which it was slightly greater. When a unit response was not obtained with one of the levels fed, an interpolation was made between a level which gave too great a response and one to which the response was not sufficient.

ANTIRACHITIC POTENCY OF THE IRRADIATED YEAST AND IRRADIATED ERGOSTEROL

An amount of yeast sufficient for both series was reserved. The potency at the beginning of Series 1 and at the end of Series 2 was 542 units per gram. Several lots of irradiated ergosterol were prepared from a stock solution and assayed. The potency of the solutions in corn oil used in the feeding trials varied from 13,300 to 16,000 units per cc. These values were the result of a quantitative assay in which a number of levels of the products were fed. The levels differed from each other, near the unit level, by 10 to 15 per cent and therefore the potency was within 15 per cent of the actual value and was not a minimum value.

FEEDING PLAN

The experimental groups consisted of 4 cows each from the herd of the Walker-Gordon Laboratory Co., Inc., Plainsboro, New Jersey, used in the production of certified milk. The groups were constituted so that the individuals of each had essentially the same milk and butterfat production records as those of the other groups and were in practically the same stage of lactation. The breed distribution was 2 Holsteins, 1 Guernsey, and 1 Jersey in each group. The ration consisted of a grain mixture, beet pulp, silage, and alfalfa with which a milk of very low vitamin D potency was produced as demonstrated by the no supplement sample, Table 1. During the course of the experiment the cows were not permitted to come into contact with direct sunlight. The two irradiated products were incorporated in the grain mixture and an amount of the mixture fed daily which supplied the number of rat units indicated in Table 1 for each group.

COLLECTION OF SAMPLES

In the case of Series 1, after 6 weeks of the experimental régime, 3 samplings were made a week apart. The cows were milked 3 times in 24 hours and therefore a sample was composited of the cream from each milking

upon the basis of production and fat content, so that the resulting sample represented the fat content of the 24-hour sample. Butter was prepared from the cream of each of the 3 samples, taken at weekly intervals, and the water and curd removed by centrifuging the liquid butter. A composite sample of the butterfats of the three 24-hour samples was then made, the amount of each of the samples used being determined upon a fat production basis. After the completion of Series 1 a sample of butterfat was obtained from the general herd which did not receive an antirachitic supplement but which received the same ration as the cows of the experiment. This sample was considered representative of a negative control group. The samples of Series 2 were collected and prepared as just described for Series 1, after the supplements had been fed for 5 weeks.

RESULTS AND DISCUSSION

The butterfats, as well as the supplies of irradiated ergosterol and irradiated yeast were assayed in two laboratories, by two groups of workers, essentially by the method described above. Different breeding rations were used in the two colonies and in Laboratory A a slightly higher percentage of calcium carbonate was employed than is prescribed for the Steenbock 2965 diet. On several occasions check readings of the line tests of one laboratory by a worker from the other showed the same conception of value for the scorings listed above. At times Laboratory A supplied animals to Laboratory B and in a few instances those of the latter laboratory were used by the former but the line test readings did not reveal any marked difference between the animals of a laboratory and those brought into it. The results of one laboratory were not consistently higher than those of the other for the two series, but in Series 1 those of Laboratory B averaged about 25 per cent higher than those of Laboratory A, whereas in Series 2 the reverse was true.

In Series 1 a smaller number of test rats per level was used than in Series 2, because as soon as it became evident that the milks were considerably below 160 units per quart, which had been mentioned as a therapeutic standard (8), Series 2 was begun and the work of the first series abandoned. However the results in Series 1 have considerable quantitative significance because a series of feeding levels was used and it was possible to designate certain levels as meeting the requirements of a unit level with a level immediately below to which the response was less and one immediately above to which the response was greater. Furthermore if the rats of both laboratories are considered as a single group the results in most instances do not differ greatly from the average for the two laboratories as presented in Table I.

In the case of Series 2, the first assay was made in July, 1932. Laboratory A used at least 6 and usually 8 or more animals at and near the unit

levels whereas Laboratory B used only 4 to 6. In November, 1932 the latter laboratory used enough additional test animals to bring the total at the important levels to 10 except in one instance when only 6 animals were used. Although the number of animals used in July or in November in Laboratory B was not sufficient for a quantitative estimate, the responses in each set of tests were of the same order and it is justifiable to combine the two sets. For Series 1, 151 test animals were employed and for Series 2, 396. Limitations of space permit the presentation of only a summary of the unit levels.

As the first step in the reinvestigation of the comparative effectiveness of irradiated yeast and irradiated ergosterol the two sources of the factor were tested as the 60,000 unit level already reported to be effective in the case of yeast (8) and in the case of irradiated ergosterol at twice this level. On account of differences in interpretation of the results of the rat assay, it is not possible to make an accurate comparison of the results of this

TABLE I
Summary of Daily Supplements to the Cows and the Antirachitic Value of the Milks Produced

DAILY SUPPLEMENT TO COWS	LABORATORY A			LABORATORY B			Average rat units per qt.
	Unit supple- ment	Test rats used at unit level	Rat units per qt.	Unit supple- ment	Test rats used at unit level	Rat units per qt.	
Series 1							
	mg.			mg.			
60,000 rat units irra- diated yeast ..	1200	7	33	1000	8	39	35
60,000 rat units irra- diated ergosterol .	1100	5-8*	35	900	8-5	43	39
60,000 rat units irra- diated ergosterol with hydroquinone	600	7	65	600	5	65	65
120,000 rat units ir- radiated ergosterol	800	5	49	600	4	65	56
No supplement	(Used 8 test animals)			(Used 7 test animals)			Less than 8
Series 2							
120,000 rat units ir- radiated ergosterol with hydroquinone .	375	3-7	104	—	—	—	104
180,000 rat units ir- radiated ergosterol	275	8-6	142	350	10	111	125
180,000 rat units ir- radiated ergosterol with hydroquinone .	275	10-10	142	325	11-6	120	130
180,000 rat units ir- radiated yeast	225	8-8	173	275	10-10	142	156
300,000 rat units ir- radiated ergosterol	275	11-9	142	275	10-10	142	142

* Refers to number of rats used on a level above and a level below that selected as a unit level.

investigation with those reported by other workers, but approximate comparisons can be made. As indicated in Table I the potencies of the butterfats produced when 60,000 units each of irradiated yeast and irradiated ergosterol were fed are essentially the same, and that of the yeast milk, 35 units per quart, less than that reported by Hess and associates (8) at the 60,000 unit level, namely 160 units per quart. It is possible that these workers used an irradiated yeast which was known to contain at least 60,000 units per daily portion yet its potency may have been actually much higher. At the 100,000 and 200,000 unit levels of irradiated ergosterol the potencies of the butterfats reported by these investigators are in only rough agreement with those obtained in this laboratory at the 120,000 and 180,000 unit levels. For the 180,000 unit level of irradiated ergosterol the results are of approximately the same order as those reported by Krauss, Bethke and Monroe (6) when 200,000 units were fed.

A graphic representation, Fig. 1, of the results displayed in Table I,

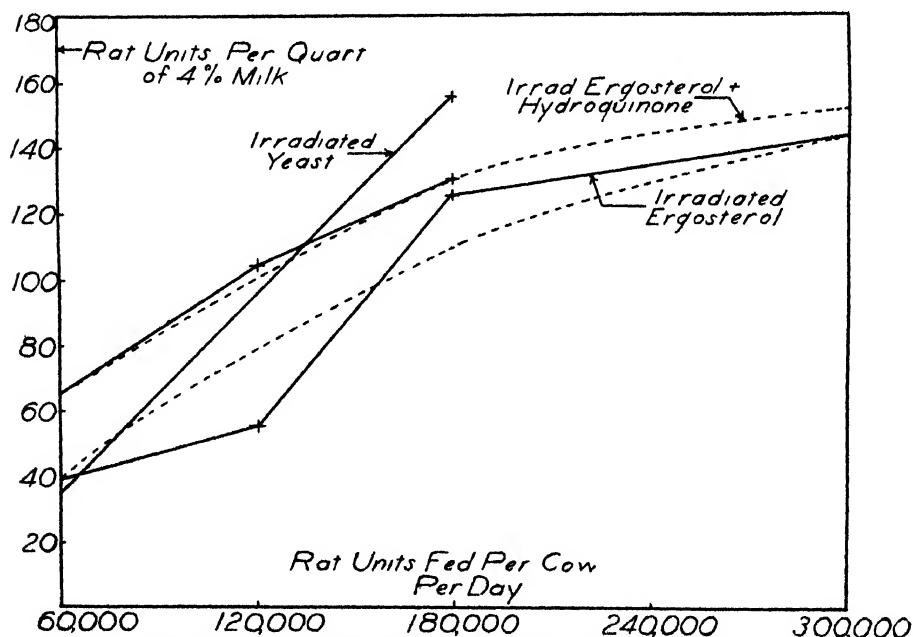


FIG. 1. The broken-line curves show the general trend of the solid-line curves which were determined by experimental values. In the case of irradiated ergosterol with hydroquinone the broken-line also serves as an extrapolation of the curve of observed values.

shows the relationship between the daily supplement to the cow and the potency of the milk when the daily allowance of the irradiated substances was increased. Although only two points are available for the irradiated yeast curve, the slope of this curve as compared with that for irradiated

ergosterol shows that the potency of the milk increases more rapidly with an increase in the amount of irradiated yeast fed than in the case of an increased allowance of irradiated ergosterol. The direction of the curve for irradiated yeast feeding also shows that a maximum milk potency is not being approached. Furthermore some commercial vitamin D milks, produced by the feeding of irradiated yeast, when assayed in this laboratory have shown a potency of as much as 200 units per quart. The general trend of the curve for irradiated ergosterol is parabolic in character and indicates that a further increase in the number of units of this product fed to the cow would not be accompanied by a marked increase in the potency of the milk.

When Hess, Light, Frey, and Gross (12) fed 450,000 units of irradiated yeast per day the potency of the milk, calculated from their data, was 975 units per quart and when the daily supplement was 1,500,000 units of irradiated ergosterol the calculated potency of the milk was 1950 units per quart. These values are considerably higher than those which would be expected if the ratio of the largest number of units fed to the cow to the number produced per quart in the present experiment prevailed at the higher levels used by Hess and associates. This ratio would predict 390 units per quart in the case of irradiated yeast and 710 units for irradiated ergosterol if the amounts used by Hess and associates were fed. The cause of this lack of agreement is not known.

It was considered possible that the greater effectiveness ascribed to irradiated yeast by Hess and co-workers (8) might be due to the protective influence of some natural antioxidant in the yeast cell. In an attempt to simulate such a condition in the use of irradiated ergosterol, 0.75 per cent of hydroquinone was introduced into the oil solution. The use of this antioxidant has been studied by Huston and Hoppert (13). The feeding of this solution at the same levels as the irradiated sterol without an added antioxidant resulted in a milk somewhat more potent at the 60,000 and 120,000 unit levels but the difference was only slight at the 180,000 unit level. Since the general trend of the curves for irradiated ergosterol without hydroquinone and of the extrapolation of the curve for irradiated ergosterol with the antioxidant indicate that a maximum is being approached, it is possible that any antioxidant properties are less effective at higher levels because other limiting factors come into play. It is of interest, Fig. 1, that the efficiency of irradiated ergosterol with hydroquinone in the production of vitamin D milk is greater at the 60,000 unit level than either irradiated yeast or irradiated ergosterol alone. At higher levels it is also more effective than irradiated ergosterol alone but less effective than irradiated yeast.

Thomas and MacLeod (7) reported irradiated yeast to be 1.5 to 3 times as effective as irradiated ergosterol and Hess and co-workers (8) placed the

effectiveness at 3 times that of the irradiated sterol in the production of vitamin D milk. In the present investigation the use of 180,000 units of irradiated ergosterol produced a milk whose potency was slightly less than that produced when 180,000 units of irradiated yeast were fed, and the ratio of the effectiveness of irradiated yeast to that of the irradiated sterol is about 1.25:1.0. Essentially the same response was obtained with 180,000 units of yeast as with 300,000 units of the sterol and the ratio of the effectiveness in this case is of the order of 2:1.

Upon the basis of an average daily production per cow of 16 quarts of milk, when 180,000 units of the antirachitic factor were consumed in the form of irradiated yeast 1.7 per cent of the factor appeared in the milk; when 300,000 rat units of irradiated ergosterol were consumed 0.7 per cent of the factor was found in the milk. For none of the other feeding levels studied was the transfer to the milk greater than 1.7 per cent.

SUMMARY

When 60,000 units were fed per cow per day, the efficiency of irradiated ergosterol in the production of vitamin D milk is approximately the same as that of irradiated yeast. The potency of the milk was 35 to 40 units per quart. At higher levels, 180,000 units per cow per day, irradiated yeast is the more efficient product, the potency of the yeast milk being of the order of 150 to 160 rat units per quart and that of the irradiated ergosterol milk 120 to 130 units. A further increase in the daily allowance of irradiated ergosterol from 180,000 to 300,000 units caused only a slight increase in the potency of the milk.

The addition of hydroquinone increases the effectiveness of irradiated ergosterol, but to a greater extent at the lower than at the higher levels of feeding.

In the case of both products less than 2.0 per cent of the units of the antirachitic factor ingested appeared in the milk.

BIBLIOGRAPHY

- (1) LUCK, E. M. The influence of diet and sunlight upon the growth-promoting and antirachitic properties of the milk afforded by a cow. *Biochem. J.* 18: 716. 1924.
- (2) GOLDING, J., SOAMES, K. M., AND ZILVA, S. S. The influence of the cow's diet on the fat-soluble vitamins of winter milk. *Biochem. J.* 20: 1306. 1926.
- (3) HART, E. B., STEENBOCK, H., TEUT, E. C., AND HUMPHREY, G. C. The influence of cod-liver oil on calcium metabolism of milking cows. *J. Biol. Chem.* 84: 359. 1929.
- (4) WACHTEL, M. The increase in volume and vitamin content of human and cow milk by means of irradiated yeast. *Munch. med. Wochschr.* 76: 1513. 1929.
- (5) STEENBOCK, H., HART, E. B., HANNING, FLORA, AND HUMPHREY, G. C. The antirachitic value of cow milk as modified by the feeding of irradiated yeast. *J. Biol. Chem.* 88: 197. 1930.

- (6) KRAUSS, W. E., BETHKE, R. M., AND MONROE, C. F. The effect of feeding irradiated ergosterol to cows on the vitamin D content of milk. *J. Nutr.* 5: 467. 1932.
- (7) THOMAS, B. H., AND MACLEOD, FLORENCE L. Increasing the vitamin D potency of cow's milk by the daily feeding of irradiated yeast or irradiated ergosterol. *Sci.* 73: 618. 1931.
- (8) HESS, A. F., LEWIS, J. M., MACLEOD, F. L., AND THOMAS, B. H. Antirachitic potency of the milk of cows fed irradiated yeast or ergosterol. *J. Amer. Med. Assoc.* 97: 370. 1931.
- (9) BILLS, C. E., HONEYWELL, E. M., WIRICK, A. M., AND NUSSMEIER, MILDRED. A critique of the line test for vitamin D. *J. Biol. Chem.* 90: 619. 1931.
- (10) DYER, F. J. The standardization of vitamin D by the line test. *Quart. J. Pharm. and Pharmacol.* 4: 503. 1931.
- (11) MCCOLLUM, E. V., SIMMONDS, N., SHIPLEY, P. G., AND PARK, E. A. Studies on experimental rickets. XVI. A delicate biological test for calcium-depositing substances. *J. Biol. Chem.* 5: 41. 1922.
- (12) HESS, A. F., LIGHT, R. F., FREY, C. N., AND GROSS, J. A study of milk, blood, and excreta of cows fed moderate and excessive amounts of irradiated yeast or ergosterol. *J. Biol. Chem.* 97: 369. 1932.
- (13) HUSTON, R. C., AND HOPPERT, C. A. The stabilizing action of hydroquinone on vitamins A and D in cod liver oil-grain mixtures. Abstracts, Div. of Biol. Chem., American Chemical Society. Meeting at Cincinnati, Ohio, Sept. 8-12, 1930.

VITAMIN STUDIES XX. THE EFFECT OF VARIOUS METHODS OF PASTEURIZATION ON THE VITAMIN B AND THE VITAMIN G CONTENT OF COW'S MILK*

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With the recognition of vitamins B and G as separate entities, it has become necessary to re-evaluate much of the previous work regarding the thermostability of the vitamin B complex. Furthermore, the methods for the biological assay of vitamins B and G have been undergoing improvement and refinement with the result that many of the recent researches on the vitamin B and G content of cow's milk have shown lack of uniformity, owing to differences in technique and in standards of biological response used by different research workers.

Aykroyd and Roscoe (1), using (winter) cow's milk as the sole source of vitamin G, reported that an intake of 3 to 6 mls. (daily) produced gains of 9 and 12 grams per week (respectively) during a four week curative period, while Hunt and Krauss (2) observed that 5 mls. of milk were necessary to furnish sufficient vitamin G to produce a weekly gain in weight of 7 to 11 grams. Samuels and Koch (3) state that 25 mls. of milk were necessary to produce optimal growth when used as the sole source of vitamin B, while 17 to 20 mls. were required to furnish sufficient vitamin G. Todhunter (4) found that 9.3 mls. per week of pasteurized milk furnished sufficient vitamin G to produce a gain of 3 grams per week during an eight week experimental period. Krauss, Erb, and Washburn (5) state that about 25 per cent of the original vitamin B in milk is destroyed during ordinary pasteurization but that vitamin G is thermostable under these conditions. These writers found that 7.5 mls. of cow's milk were necessary to furnish one Sherman unit of vitamin B, while one unit of vitamin G was furnished by 5 mls. of the same milk. MacLeod, Brodie, and Maleloon (6) reported that they found that the vitamin B and the vitamin G content of milk tended to remain fairly constant throughout the year and that the average content of vitamins B and G was 0.1 and 0.3 units (respectively) per gram of milk.

The studies reported in the present paper were initiated with the hope of obtaining further information regarding the vitamin B and vitamin G content of milk obtained from cows fed under carefully controlled conditions and to study the thermostability of these vitamins when subjected to various methods of pasteurization.

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EXPERIMENTAL

The preparation, composition, and handling of the basal diet used throughout this investigation were the same as that reported in a previous publication from this laboratory (7).

The vitamin supplement was prepared by percolating acidified commercial ethyl alcohol (5 mls. of concentrated HCl per liter of alcohol) through dried brewer's yeast as long as any color was removed from the yeast by this solvent. The percolate was concentrated under diminished pressure until it possessed the consistency of a thick syrup. The concentrated extract was cooled, placed in a separatory funnel and, after being diluted with one-half its volume of distilled water, was shaken with three separate portions of ethyl ether in order to remove the fatty materials. The fat-free extract was again concentrated under diminished pressure until 1 ml. of this concentrate represented the extract from approximately 20 grams of yeast. The excess acid was partially neutralized (pH of approximately 5.0) by carefully adding small quantities of a 10 per cent solution of sodium hydroxide. The concentrate was then diluted with alcohol to such a volume that 0.1 ml. of the resulting solution represented 1 gram of the original yeast. This extract was maintained at a sub-zero temperature for twenty-four hours after which it was rapidly filtered through a small Buchner funnel by means of suction, and preserved in a refrigerator until used.

The vitamin G supplement was prepared from dried baker's yeast by moistening the yeast with a saturated solution of sodium bicarbonate until a slightly alkaline product resulted. The alkalinized yeast was autoclaved for six hours at 10 pounds pressure, after which it was dried and pulverized.

The milk used throughout the investigation was purchased from the college creamery. It was produced by the college herd and was of "certified" grade. The college herd received a uniform diet composed of a complex grain mixture, supplemented by alfalfa hay, ensilage and salt. The quantity of grain-mixture fed to each cow was proportional to the body weight of the animal and to the amount of milk produced, while each cow received eight pounds of alfalfa hay and twenty-two pounds of ensilage daily. The milk was produced under the most favorable sanitary conditions, and was cooled to a temperature of 36 to 40° F. immediately after milking. The raw milk was tested for its vitamin B and vitamin G potency as produced and again after each of four different heat treatments. The four different heat treatments employed were as follows: (I) ordinary pasteurization, (II) pasteurization under reduced pressure, (III) pasteurization with aeration, and (IV) by boiling for ten minutes.

In the first method (I) of pasteurization the milk was placed in an Erlenmeyer flask and stoppered with a one-hole rubber stopper, through which projected a thermometer. The flask of milk was placed in a water-

bath and the temperature of the milk was raised to 62–63° C. and maintained at this temperature for thirty minutes.

The second method (II) was very similar to the first except that a two-hole stopper containing a thermometer and a glass stopcock was used. When the temperature of the milk had reached 62–63° C., the stopcock was connected to a water pump and the pressure in the flask was reduced until the milk began to boil sufficiently to fill the flask with froth. The stopcock was then closed and the milk was maintained at this temperature for thirty minutes.

In the third method (III) a two-hole stopper containing a thermometer and a urea aeration tube was employed. After the milk had been heated to a temperature of 62–63° C., the aeration tube was connected to a water pump and the flow of water was so adjusted that a steady stream of air bubbles was drawn through the milk during the thirty minutes pasteurization period.

In the fourth method (IV) of pasteurization, the milk was placed in a round bottomed flask, which in turn was connected to a reflux condenser. The milk was heated to boiling on an electric hot plate and was maintained at this temperature for ten minutes.

Equal portions of fresh raw milk were pasteurized by the above methods twice weekly (Tuesday and Saturday) during the test period. Immediately after the pasteurizing treatment, each sample of milk was cooled rapidly by means of cold water, and was stored in an electric refrigerator until used. A corresponding sample of untreated milk was similarly preserved and was fed as a positive control.

Rats 20 to 21 days of age and weighing from 39 to 45 grams were placed in individual metal cages provided with raised screen bottoms, and were fed liberal quantities of the basal diet. The animals were weighed at weekly intervals, at which time records were made of food consumption and physical condition. After the animals began to lose weight (usually from 12 to 20 days after being placed on the diet), they were divided into groups, care being taken to compensate so far as possible for both sex and litter variations. At this time the basal diet of each animal, excepting that of those retained as negative controls, was supplemented daily by definite quantities of milk, of the vitamin B concentrate or of the vitamin G fraction, either alone or in some definite combination. Each supplement to the basal diet was fed daily in separate containers for a period of at least six weeks, unless the test animal died previous to the completion of the experiment.

The first part of the investigation was restricted to testing the vitamin B concentrate, the vitamin G fraction, and the raw milk for their vitamin B complex potency. To do this, a total of 36 depleted animals was used. Six of these animals were retained on the unsupplemented basal diet to serve

as negative controls. Another group of six animals received the basal diet and, in addition, a daily supplement of 0.1 ml. of the vitamin B concentrate. A third group of six animals received (daily) 0.3 grams of autoclaved yeast as a supplement to the basal diet. A fourth group of six animals received both the vitamin B concentrate and the vitamin G fraction as supplements to the basal diet. The twelve remaining animals were divided into six groups of two animals each, and the rats in the respective groups received, as a supplement to the basal diet, 1, 2, 3, 4, 6, and 8 mls. of raw milk (V) daily. The responses made by these groups of animals are given in Table 1 and Chart 1.

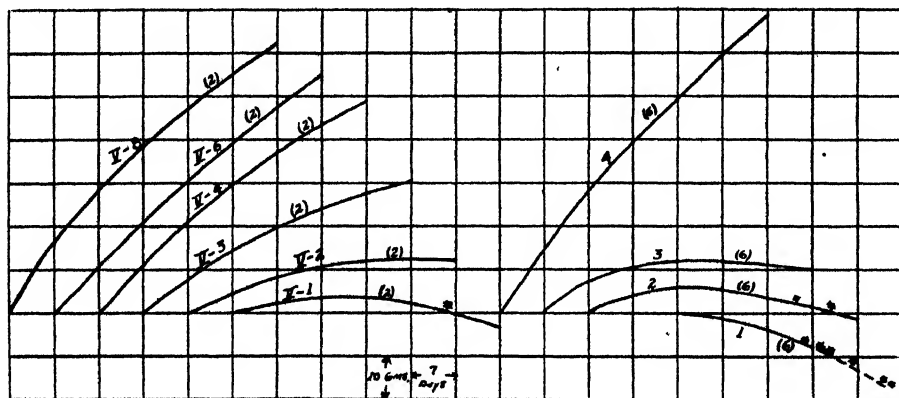


CHART 1

Showing the average growth responses made by the various groups of animals receiving the basal diet unsupplemented (Curve 1); supplemented with vitamin B concentrate (Curve 2); supplemented by the vitamin G fraction (Curve 3); supplemented by both vitamins B and G (Curve 4); and supplemented by definite quantities of unpasteurized milk (Curves V-1 to V-8). The "V" curves represent growth responses made by animals receiving milk as the only source of vitamins B and G. The number immediately following the "V" indicates the quantity of milk fed. The number in parenthesis indicates the number of animals considered. The asterisk indicates the death of an animal.

The data obtained showed that 3 mls. of the unpasteurized milk were sufficient to produce a gain of 30 grams during a six-week experimental period, when used as the sole source of the vitamin B complex. In consequence of this fact, a 3 mls. daily dosage was chosen as the amount to be fed in the study relative to the vitamin B and the vitamin G content of this milk before and after the several methods of pasteurization. Groups of animals (varying from 3 to 12 animals per group) were fed 3 mls. daily of one of the five types of milk to be tested, alone, and in combination with the vitamin B concentrate and the vitamin G fraction. An outline of this phase of the investigation, together with the resultant growth are given in Tables 1 and 2, and in Chart 2.

TABLE 1

Showing the quantities of the vitamin supplements used, the number of animals considered, their average initial weight, average weight at end of depletion period, average weekly gain, and average weekly food consumed

DIET NO.	MILK SUPPLEMENT	QUANTITY OF MILK FED	VITAMIN B CONCENTRATE FED	VITAMIN G FRACTION FED	NO. OF RATS CONSIDERED	AVERAGE INITIAL WEIGHT	AVERAGE WEIGHT AT END OF DEPLETION PERIOD	GAIN						AVERAGE WEEKLY FOOD CONSUMPTION
								1st week	2nd week	3rd week	4th week	5th week	6th week	Total
306	.	mls.	mls.	gms.		gms.	gms.	gms.	gms.	gms.	gms.	gms.	gms.	gms.
"	"		0.1		6	42	49	-3	-1	-3	-6	-1	-2	0
"	"				6	41	48	7	4	2	0			13
"	"			0.3	6	41	49	3	3	0	-2			21
"	"		0.1	0.3	6	42	48	15	13	11	10	10	8	67
"	V	1			2	41	49	3	1	0	-1		-3	32
"	V	2			2	41	48	5	3	3	2		-2	17
"	V	3			2	44	51	7	7	5	4	1	-1	19
"	V	4			2	42	48	11	10	9	7	6	2	30
"	V	6			2	41	49	11	10	10	9	8	5	24
"	V	8			2	43	50	16	12	10	9	8	7	31
"	V	3			12	43	50	8	5	4	4	3	2	36
"	V	3	0.1		6	44	50	8	10	6	7	6	6	22
"	V	3		0.3	6	43	50	6	9	9	5	2	4	25
"	I	3			11	43	51	5	3	4	3	3	2	35
"	I	3	0.1		3	42	50	8	7	8	5	5	4	20
"	I	3		0.3	3	43	51	6	5	5	4	4	3	23
"	II	3			11	43	51	3	3	3	2	3	3	24
"	II	3	0.1		3	41	48	6	6	5	4	3	2	16
"	II	3		0.3	3	42	49	6	6	4	4	3	3	24
"	III	3			10	41	48	4	4	4	2	5	3	27
"	III	3	0.1		3	43	50	8	7	6	3	3	3	22
"	III	3		0.3	3	43	51	7	7	6	6	4	3	20
"	IV	3			11	42	50	6	6	3	5	3	2	23
"	IV	3	0.1		3	43	51	7	7	6	7	5	5	22
"	IV	3		0.3	3	42	49	6	7	5	7	6	4	37
"	IV	3			3	42	49	6	7	5	7	6	4	24

TABLE 2

Showing the average gains in weight in grams made by the several groups of animals receiving 3 mls. daily of the various milks, and milks supplemented by the Vitamin B concentrate and the Vitamin G fraction, respectively

	UNPASTEURIZED MILK (v)	PASTEURIZED BY USUAL METHOD (i)	PASTEURIZED UNDER PARTIAL VACUUM (ii)	PASTEURIZED WHILE AERATED (iii)	BOILED FOR 10 MINUTES (iv)
Milk alone	26 gms. (100%)	20 gms. (77%)	16 gms. (62%)	21 gms. (81%)	22 gms. (85%)
Milk with vitamin B	43 gms. (100%)	37 gms. (86%)	27 gms. (63%)	34 gms. (79%)	37 gms. (86%)
Milk with vitamin G	35 gms. (100%)	27 gms. (77%)	26 gms. (74%)	30 gms. (86%)	34 gms. (97%)

For convenience, the data are also expressed in terms (percentage) of the responses made by groups of animals receiving the untreated milk, alone and similarly supplemented. It is realized that values obtained by this method of calculation are only approximations. More accurate results could have been obtained if definite quantities of each milk could have been fed to produce a unit gain in weight.

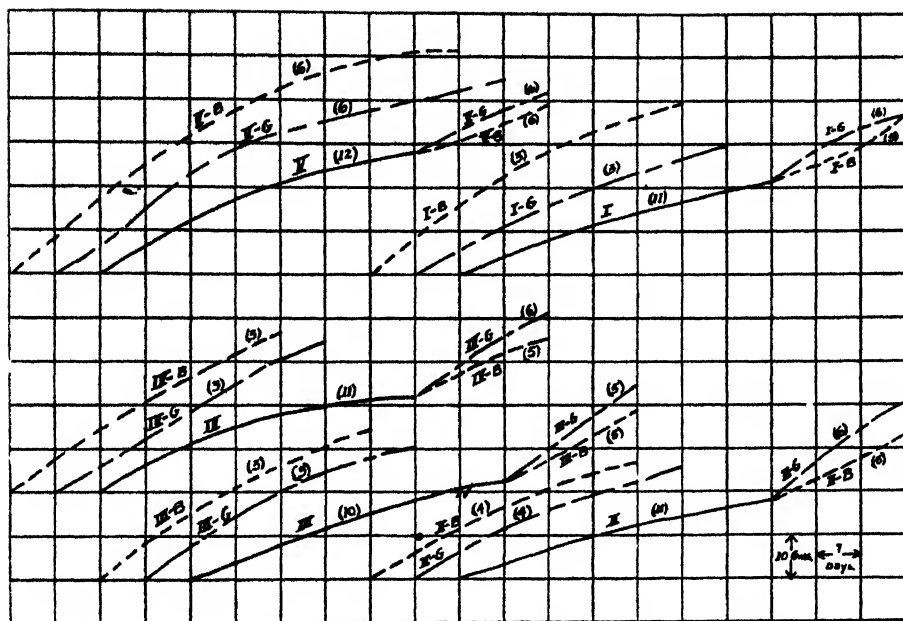


CHART 2

Showing the average growth responses made by groups of animals receiving 3 mls. daily of the various milks, alone and in combination with the vitamin B concentrate and the vitamin G fraction, respectively. The Roman numeral indicates the method of pasteurization, the letter indicates the additional supplement added, and the number in parentheses indicates the number of animals considered.

- I = ordinary pasteurization
- II = pasteurization under partial vacuum
- III = aerated while pasteurized
- IV = pasteurized by boiling for 10 minutes
- V = unpasteurized
- B = vitamin B concentrate
- G = vitamin G fraction

Those groups which received one of the five samples of milk unsupplemented by either the vitamin B concentrate or the vitamin G fraction, and which survived a seven-week experimental period, were divided into two groups of approximately equal number. The animals of one group were given, in addition to the usual milk supplement, 0.1 ml. of the vitamin B concentrate daily, while the animals of the other group received 0.3 grams of the vitamin G fraction daily. These extra supplements were fed for three additional weeks. The results obtained by adding further supplements at this stage of the investigation are shown by the extended growth curves (broken lines) in Chart 2.

DISCUSSION

While the experimental data here presented are compiled from the records of 120 animals, a considerably larger number of animals was used

in the investigation. These data are representative of those obtained as a result of three series of tests carried on at different seasons of the year. The first series of tests was begun in June, while the second and third series were started in October and March, respectively. Since the responses made by corresponding groups of animals during the three seasons were so nearly identical, the data have been presented in a composite form, using the records of only those animals which reacted most typically in their respective groups. Records of experimental animals which were improperly depleted of their vitamin stores and of those animals which were known to have resorted to any degree of coprophagy during the test period were eliminated. Efforts were made to use experimental animals of approximately the same initial weight in the various groups and during the three series of tests. As has been previously stated, these animals were distributed among the several groups so as to compensate for both litter and sex variations. A majority of the animals which were placed on the basal diet, for the depletion period, gained from 5 to 8 grams during the first 9 to 14 days, after which their weight became stationary, and in many cases a slight loss in weight ensued. It was found to be undesirable to continue the depletion period for more than 20 to 21 days because of the high incidence of spontaneous re-growths due to coprophagy, which is frequently practiced and observed among over-depleted rats receiving a basal diet of this type. Growth responses made by such animals are unreliable as indices of the vitamin B or the vitamin G potency of a food material.

The control animals which received the basal diet unsupplemented (Curve 1, Chart 1) continued to decline in weight and a majority of them were dead before the end of the fourth week of the experimental period. Some manifestations of beriberi were usually observed in each case, but the paralytic symptoms were more pronounced among those animals which survived the longest, unless such animals were practicing some degree of coprophagy.

The depleted animals which received the basal diet supplemented by the vitamin G fraction (Curve 2, Chart 1) made a slight response in growth during the first two weeks in which the supplement was fed, and then showed a gradual loss in weight until the end of the experiment. Most of these animals showed marked paralytic symptoms, especially during the sixth week, and a number of them died during this time.

Animals receiving the basal diet, supplemented by daily additions of the vitamin B concentrate (Chart 1, Curve 3), made a slow but definite growth response for the first three weeks, which was followed by a slight loss in weight during the following three weeks. By this time most of the animals were beginning to manifest some of the symptoms usually ascribed to vitamin G deficiency.

Those animals which received the basal diet supplemented by both the vitamin B concentrate and the vitamin G fraction (Curve 4, Chart 1) made an average gain in weight of 11 grams per week during the six-week period. These animals appeared normal in all respects with the exception of the abnormally dry condition of their skin and hair.

Curves V-1 to V-8 (Chart 1) show the growth response made by animals receiving varying quantities of the unpasteurized milk as a supplement to the basal diet. The quantities of milk fed daily were 1, 2, 3, 4, 6, and 8 mls., respectively. Although 1 or 2 mls. of milk daily had a definite supplemental effect on the basal diet, the animals which received these quantities of milk frequently manifested symptoms of beriberi. When the milk supplement was increased to 3 mls. daily, a fairly uniform gain in weight resulted throughout the six-week period, and none of the animals showed characteristic symptoms of vitamin B deficiency. While still greater daily allotments of milk resulted in more marked growth responses and further improvements in the general appearance of the animals, it was believed that a growth response similar to that produced by 3 mls. of milk daily would be the most accurate in evaluating the vitamin B and the vitamin G content of milk which had been pasteurized by any one of the several methods described.

Curve V (Chart 2) shows the growth response made by a larger group of animals which received (daily) 3 mls. of unpasteurized milk as a supplement to the basal diet. These animals made an average gain of 4 grams per week, and appeared to be in a fairly thrifty condition throughout the experiment. When the basal diet was further supplemented by either the vitamin B concentrate or the vitamin G fraction, in addition to the 3 mls. of unpasteurized milk, further growth resulted in each case (Curves V-B and V-G), but the vitamin B concentrate excelled the vitamin G fraction as a growth producing supplement under these conditions.

Curve I (Chart 2) shows the growth response made by a group of animals receiving the basal diet supplemented daily by 3 mls. of milk pasteurized by ordinary pasteurization (Method I). These animals showed an average gain of approximately 3 grams per week during the 6-week period. Additional growth resulted when the diet was further supplemented by the vitamin B concentrate and by the vitamin G fraction (Curves I-B and I-G), but here again the vitamin B concentrate excelled in supplementing value.

Curve II (Chart 2) shows the average growth response made by a group of 11 animals that received (daily) 3 mls. of milk which had been pasteurized under a partial vacuum (Method II). The average growth rate in this case was less than 2.7 grams per week, which is considerably less than the gains made by those animals which received the unpasteurized milk under similar conditions (Curve V). When calculated on the basis of

growth responses produced before and after pasteurization, only 62 per cent of the original vitamin B complex was retained by the milk after being pasteurized in this manner. In this case the vitamin B concentrate proved to have a marked supplemental value when fed in addition to the milk supplement (Curve II-B), while additions of the vitamin G fraction resulted in equally definite increments of growth (Curve II-G).

We are unable to offer any explanation as to why milk pasteurized by this method contains less of these vitamins than similar milk pasteurized in the usual manner (Method I). In fact, these findings were contrary to our expectations. Since similar differences were observed in three series of experiments carried on during different seasons of the year, it appears that the results are beyond the realm of chance. Some attempts were made, therefore, to arrive at a plausible explanation for the apparent destruction or inactivation of these vitamins, but no definite conclusion could be drawn from the data obtained. It does not seem probable that such destruction could be the result of any slight change in hydrogen-ion concentration of the milk as the result of the partial removal of the dissolved gases while under partial vacuum; yet this is a phase of the problem that we hope to study as soon as time will permit.

The group of animals which received the milk that had been aerated during the process of pasteurization (Method III) made an average gain of 3.5 gms. per week (Curve III, Chart 2). While these results indicate that some destruction of the vitamin B complex had taken place, this milk compared favorably with that produced by the usual method of pasteurization (Curve I). When this diet was further supplemented by either vitamin B or vitamin G fractions, additional responses in growth were obtained (Curves III-B and III-G). Of the two factors, the vitamin B concentrate proved slightly superior as a growth stimulator under these conditions.

When raw milk, which had been boiled for 10 minutes under a reflux condenser, was fed to a group of rats as a supplement to the basal diet, it was found to have retained the greater portion of its original vitamin B complex content (Curve IV, Chart 2). In fact, this milk appears slightly superior in this respect to milks pasteurized by either Methods I, II or III. Those groups of animals which received further dietary supplements in the form of either vitamin B concentrate or vitamin G fraction grew at an increased rate (Curves IV-B and IV-G). In this dietary combination the vitamin B concentrate proved more effective in stimulating additional growth than did the vitamin G fraction.

When each of the five groups of animals which had been receiving the five different milks as the sole supplement to the diet (Groups I, II, III, IV and V) were divided at the end of the seventh experimental week, one half of each group being given daily allotments of the vitamin B concen-

trate and the other half of each group being given the vitamin G fraction in addition to the usual daily allotment of the respective milks, some interesting growth responses were observed (see broken line extensions of Curves I, II, III, IV and V). At this stage of the experiment, the vitamin G supplement proved to be the most effective growth-stimulator in every case. These results are not in agreement with those which had been obtained by means of similar groups of animals of less mature age, during the preceding weeks. But the consistency of growth-trends in this connection is sufficient at least to suggest that the relative vitamin B and vitamin G requirements of the rat vary with variations in stage of maturity.

SUMMARY

1. Experiments are described in which raw certified milk, produced by the college dairy herd, was fed to rats with and without supplementation with potent preparations of vitamins B and G.

2. At three different periods during the year these experiments were repeated, at which times the raw milk was pasteurized by four methods, *viz.*, (1) ordinary pasteurization at 62–63° C. for 30 minutes; (2) same as (1) except that pasteurization was conducted under reduced pressure; (3) same as (1) except that the milk was aerated; and (4) boiled for 10 minutes under a reflux condenser.

3. The pasteurized milks were also fed to rats in the presence and in the absence of potent preparations of vitamins B and G and comparisons were made with raw milk from which the pasteurized milks were made.

4. It was found that the raw milk contained appreciable quantities of vitamins B and G and that 3 mls. per day were sufficient to furnish at least one Sherman unit of each of the vitamins.

5. It was found that vitamin B was the limiting factor when 3 mls. of raw milk were fed as the sole source of vitamins B and G but the data indicate that the B potency was not limited to the extent that has been reported by certain investigators.

6. It was observed that the vitamin B and G potency of the raw milk was remarkably constant throughout the year.

7. Three separate sets of experiments showed greater loss of vitamin B and vitamin G when the milk was pasteurized under diminished pressure. This was contrary to expectations and will be investigated in the near future.

8. Some loss of vitamins B and G occurred in all methods of pasteurization but less destruction occurred when milk was boiled for 10 minutes under a reflux condenser.

9. Although the maximum destruction of either vitamin B or vitamin G, as a result of any one of the four methods of pasteurization, was about 38 per cent (calculated from differences in growth response), the destruction

of these vitamins under carefully controlled plant operation need not be as great as indicated above.

10. It is suggested that the relative requirements for vitamin B and vitamin G by the rat vary with the age of the rat, the evidence suggesting that the requirement for vitamin B is greater in the young rat and that the need for vitamin G seems to be greater as the rat matures.

REFERENCES

- (1) AYKROYD, W., AND ROSCOE, M. The distribution of vitamin B₂ in certain foods. *Biochem. J.* **23**: 483-497. 1929.
- (2) HUNT, C. H., AND KRAUSS, W. E. The relative antineuritic and antipellagagic potency of cow's milk. *J. Biol. Chem.* **79**: 733-738. 1928.
- (3) SAMUELS, L. H., AND KOCH, F. C. The relative quantities of heat-stable and heat-labile fractions of vitamin B in raw and evaporated milk. *J. Nutrition* **5**: 307-324. 1932.
- (4) TODHUNTER, E. N. A comparison of the vitamin G value of pasteurized milk, evaporated milk, and eggs. *J. Am. Dietetic Assoc.* **3**: 42-46. 1932.
- (5) KRAUSS, W. E., ERB, J. H., AND WASHBURN, R. G. The effect of pasteurization on some of the nutritive properties of milk. *Ohio Agric. Exp. Sta. Bulletin* 518. 1933.
- (6) MACLEOD, F. L., BRODIE, J. B., AND MACLOON, E. R. The vitamin A, B, and G content of milk throughout the year. *J. DAIRY SCIENCE* **15**: 14-22. 1932.
- (7) GUERRANT, N. B., AND DUTCHER, R. ADAMS. The assay of vitamins B and G as influenced by coprophagy. *J. Biol. Chem.* **93**: 225-235. 1932.

AMERICAN DAIRY SCIENCE ASSOCIATION

Annual Meeting

Ithaca and Geneva, New York, June 26 to 28, 1934

OUTLINE OF PROGRAM

Monday, June 25, Ithaca

Registration and assignment of rooms. Willard Straight Hall.

2:00 P. M.—Dairy cattle judging conference. Animal Husbandry Building.

2:00 P. M.—Dairy products judging conference. Dairy Industry Building.

Tuesday, June 26, Ithaca

Registration and assignment of rooms. Willard Straight Hall.

9:30 A. M.—Address of Welcome. Dairy Industry Building, Room 218.

9:45 A. M.—Business Meeting. Dairy Industry Building, Room 218.

10:15 A. M.—General Session. Dairy Industry Building, Room 218.

12:00 M. —Luncheon.

1:30 P. M.—Production Section. Animal Husbandry Building, Room A.

1:30 P. M.—Manufacturing Section. Dairy Industry Building, Room 218.

1:30 P. M.—Extension Section. Animal Husbandry Building, Room C.

3:30 P. M.—Recreation and picnic supper. Taughannock Falls Park.

Wednesday, June 27, Ithaca

9:00 A. M.—Production Section. Animal Husbandry Building, Room A.

9:00 A. M.—Manufacturing Section. Dairy Industry Building, Room 218.

9:00 A. M.—Extension Section. Animal Husbandry Building, Room C.

12:00 M. —Luncheon.

1:30 P. M.—Production Section. Animal Husbandry Building, Room A.

1:30 P. M.—Manufacturing Section. Dairy Industry Building, Room 218.

4:00 P. M.—Business Meeting. Dairy Industry Building, Room 218.

6:30 P. M.—Annual Banquet. Willard Straight Hall. Address by Professor George F. Warren.

Thursday, June 28, Geneva

Check out and leave Ithaca for Geneva before 8:15 A. M. Transportation will be arranged for those without automobiles.

9:45 A. M.—Address of Welcome. Jordan Hall, Room A.

10:00 A. M.—Business Meeting. Jordan Hall, Room A.

10:30 A. M.—General Session. Jordan Hall, Room A.

12:00 M. —Luncheon.

1:30 P. M.—Production Section. Jordan Hall, Room A.

1:30 P. M.—Manufacturing Section. Jordan Hall, Room B.

3:30 P. M.—Entertainment.

Final Adjournment.

Tuesday morning, June 26, Ithaca
Dairy Industry Building, Room 218

ADDRESS OF WELCOME

9:30 A. M.

DEAN CARL E. LADD

BUSINESS MEETING

9:45 A. M.

Report of Secretary-Treasurer
Announcements by the President
Appointment of committees
Announcements of Program Committee

GENERAL SESSION

10:15 A. M.

R. B. STOLTZ, *Chairman*

- G1—Progress in the study of the hormones concerned with milk secretion. C. W. Turner, University of Missouri.
- G2—Sugars and lactose formation. W. E. Petersen and W. R. Brown. University of Minnesota.
- G3—Removal of salts from whey protein for use in infant feeding. P. D. Watson, Bureau of Dairy Industry.
- G4—The vitamin-A content of butterfat from Ayrshire, Guernsey, Holstein, and Jersey cows: I. Biological response. II. Relationships between yellow color, carotene and vitamin A. W. E. Kraus and T. S. Sutton, Ohio Agricultural Experiment Station.
- G5—Milk marketing agreements and licenses. J. B. Parker, Bureau of Dairy Industry.
- G6—The Michigan plan of dairy products utilization campaigns. A. C. Baltzer, Michigan State College.

Tuesday afternoon, June 26, Ithaca

(Three sections, Production, Manufacturing, and Extension, will meet concurrently.)

PRODUCTION SECTION

1:30 P. M.

Animal Husbandry Building, Room A

H. O. HENDERSON, *Chairman*

- P1—Effect of temperature of artificial drying on digestibility and availability of nutrients in pasture herbage. R. E. Hodgson, J. C. Knott, R. R. Graves, and H. K. Murer, Bureau of Dairy Industry and State College of Washington.
- P2—Increased hay feeding for dairy cows. C. F. Monroe and Harold Allen, Ohio Agricultural Experiment Station.
- P3—Feeding dairy cattle on alfalfa hay and irrigated pasture without grain. H. S. Willard, University of Wyoming.
- P4—A comparison of blue grass pasture with pastures of sweet clover seeded with oats or oats and field peas. R. A. Ackerman and H. O. Henderson, West Virginia University.

- P5—Pasture fertilization results. R. H. Lush, Louisiana Agricultural Experiment Station; and J. L. Fletcher, Southwestern Louisiana Institute.
- P6—Early-cut nitrogen fertilized timothy hay, vs. alfalfa hay for milk production. G. W. Salisbury and F. B. Morrison, Cornell University.
- P7—The nutritive value of the proteins of alfalfa hay and other feeding stuffs. K. L. Turk, F. B. Morrison, and L. A. Maynard, Cornell University.
- P8—Pasture Committee report. R. B. Becker, C. B. Bender, G. Bohstedt, I. R. Jones, and R. H. Lush, Chairman.

MANUFACTURING SECTION

1:30 P. M.

Dairy Industry Building, Room 218

HAROLD MACY, *Chairman*

- M1—The influence of feed and the individuality of cows on the susceptibility of milkfat to oxidation. J. L. Henderson and C. L. Roadhouse, University of California.
- M2—Studies of the causes of oxidized flavors in milk. L. M. Thurston and W. Carson Brown, West Virginia University.
- M3—Another source of "oxidized" flavors of milk. E. S. Guthrie and H. J. Brueckner, Cornell University.
- M4—Seasonal variations in the lipase content of milk. J. L. Hileman and Eleanor Courtney, Dairymen's League Cooperative Association.
- M5—The lecithin content of milk and its relation to abnormal milk. B. E. Horrall, Purdue University.
- M6—Prevention of cheesy flavors in unsalted butter. E. O. Herreid and H. Macy, University of Minnesota.
- M7—The use of dichlorofluorescein in the determination of chlorides in milk and dairy products. H. G. Lindquist, Massachusetts State College.
- M8—Measuring oxidation-reduction potentials. R. J. Ramsey, University of Illinois.

EXTENSION SECTION

1:30 P. M.

Animal Husbandry Building, Room C

J. W. LINN, *Chairman*

- E1—Putting proved sire information to work. J. C. Nisbet, Hoard's Dairyman.
- E2—Use of information from sire record books. Earl Schultz and Floyd Johnston, Iowa State College.
- E3—Organizing a sire campaign. S. J. Brownell, Cornell University.
- E4—Increasing the value of dairy herd improvement association records. J. F. Kendrick, Bureau of Dairy Industry.
- E5—Getting milk and fat production costs in Dairy Improvement Association herds. E. J. Perry, New Jersey Agricultural College.
- E6—Progress reports on comparison of lactation and Cow Testing Asso-

ciation yearly records. G. M. Harris, Jay L. Lush, and E. N. Shultz, Iowa State College.

E7—The essentials of a constructive breeding program. E. E. Heizer, Ohio State College.

E8—Does continuous testing pay? J. C. McDowell, Bureau of Dairy Industry.

Wednesday morning, June 27, Ithaca

(Three sections, Production, Manufacturing, and Extension, will meet concurrently.)

PRODUCTION SECTION

9:00 A. M.

Animal Husbandry Building, Room A

H. O. HENDERSON, *Chairman*

- P9—The development of nutritional anemia in dairy calves. C. E. Knoop, W. E. Kraus, and R. G. Washburn, Ohio Agricultural Experiment Station.
- P10—The iron and copper content of milk throughout the season as related to anemia development in rats. W. E. Kraus and R. G. Washburn, Ohio Agricultural Experiment Station.
- P11—Carotene and vitamin A in dairy feeds. E. A. Kane, Bureau of Dairy Industry.
- P12—Carotene and vitamin A in the nutrition of dairy calves. H. T. Converse, Bureau of Dairy Industry.
- P13—The value of certain home-grown roughages and concentrates in maintaining a high vitamin A value of butterfat. J. H. Hilton, J. W. Wilbur, and S. M. Hauge, Purdue University.
- P14—The effect of age and phosphorus intake on inorganic phosphorus content of whole blood of dairy heifers. A. H. Van Landingham and H. O. Henderson, West Virginia University.
- P15—Influence of dry period and of mineral supplement on subsequent lactation. P. T. Dix Arnold and R. B. Becker, Florida Agricultural Experiment Station.
- P16—Fluorine ingestion, its toxicity and pathology in dairy cattle. P. H. Phillips, University of Wisconsin.
- P17—The influence of fat in the milk on the rate of evacuation of the calf's stomach. D. L. Espe and C. Y. Cannon, Iowa State College.
- P18—Ovarian development and reaction to experimental administration of gonadotrophic hormones in the calf. L. E. Casida, I. W. Rupel, and A. B. Chapman, University of Wisconsin.
- P19—The theoretical value of grain in the dairy ration. C. Y. Cannon and D. L. Espe, Iowa State College.
- P20—The feeding value of cane molasses when incorporated in grain mixtures for dairy cows. G. Bohstedt, I. W. Rupel, B. H. Roche, and P. E. Newman, University of Wisconsin.

MANUFACTURING SECTION

9:00 A. M.

Dairy Industry Building, Room 218

HAROLD MACY, *Chairman*

- M9—The color imparted to coffee by cream treated in various ways. Randall Whitaker, National Dairy Products Corporation.

- M10—The use of citric acid and sodium citrate in buttermaking. Hugh L. Templeton, University of Wisconsin.
- M11—Neutralizing high acid cream for buttermaking. E. L. Fouts and Earl Weaver, Oklahoma A. and M. College.
- M12—The application of the microscope to the biological examination of butter. J. A. Nelson, Montana State College.
- M13—Bacteriological and chemical studies of creamery water supplies. H. Macy and F. E. Nelson, University of Minnesota.
- M14—Fat test variations in weigh tanks. J. C. McCan, New Jersey Agricultural Experiment Station.
- M15—Measurement of the coefficient of heat transfer of metals used in dairy equipment. L. C. Thomsen and Theodore R. Coker, University of Wisconsin.
- M16—The effect of heat transfer rates upon some properties of milk and cream. J. C. Marquardt and A. C. Dahlberg, New York Agricultural Experiment Station.
- M17—Change in pH of the medium by the growth of yeasts. E. H. Parfitt, Purdue University.
- M18—Influence of the oxidation-reduction potential of the medium upon the growth of yeasts and molds. E. H. Parfitt, Purdue University.
- M19—Sedimentation in homogenized milk. D. A. Charles and H. H. Sommer, University of Wisconsin.
- M20—Some considerations in the homogenization of milk. C. J. Babcock, Bureau of Dairy Industry.

EXTENSION SECTION

9:00 A. M.

Animal Husbandry Building, Room C

J. W. LINN, *Chairman*

- E9—Adjusting the dairy extension program to new conditions. J. A. Arey, North Carolina State College.
- E10—Planning county dairy programs. H. R. Searles, University of Minnesota.
- E11—The use of contracted acres to reduce cost of producing dairy products. D. M. Seath, Kansas State College.
- E12—Suggestions for register changes in any dairy breed. E. S. Savage, Cornell University.
- E13—An exhibit of ideas. Floyd Johnston, Iowa State College.
- E14—Presenting feed record analyses. W. T. Randall, Cornell University.
- E15—A departmental short course for milk inspectors. J. I. Keith and Earl Weaver, Oklahoma A. and M. College.
- E16—Frequent quizzes in teaching dairy elements. E. L. Fouts and Earl Weaver, Oklahoma A. and M. College.
- E17—Making, packaging, and curing cheddar cheese in sealed containers. H. L. Wilson, Bureau of Dairy Industry.
- E18—The Vogt method of manufacturing flake buttermilk. E. S. Guthrie, Cornell University.

Wednesday afternoon, June 27, Ithaca

(Two sections, Production and Manufacturing, will meet concurrently.)

PRODUCTION SECTION

1:30 P. M.

Animal Husbandry Building, Room A

H. O. HENDERSON, *Chairman*

- P21—Should milk yield be corrected for age of cow or size of cow? W. L. Gaines, University of Illinois.
- P22—A tentative method for correcting milk yield for gains or losses in body weight. J. W. Rupel, University of Wisconsin.
- P23—Significance of body weights in feeding experiments with milk cows. A. H. Kuhlman and Earl Weaver, Oklahoma A. and M. College.
- P24—The cow's individuality and the herd management as causes of differences in production. Mogens Plum, Iowa Agricultural Experiment Station.
- P25—The distribution of nitrogen in milk as affected by the level of protein feeding. A. E. Perkins, Ohio Agricultural Experiment Station.
- P26—The influence upon milk production of three different planes of protein intake. S. H. Work and E. S. Harrison, Cornell University.
- P27—Milk production with excessive amounts of cottonseed meal. A. H. Kuhlman and Earl Weaver, Oklahoma A. and M. College.
- P28—A study of bull indexes. E. S. Savage and Margarete Altmann, Cornell University.
- P29—Production factors. J. P. LaMaster, Clemson Agricultural College.
- P30—The effect of alfalfa hay on milk flavor. Earl Weaver, Oklahoma A. and M. College.

MANUFACTURING SECTION

1:30 P. M.

Dairy Industry Building, Room 218

HAROLD MACY, *Chairman*

- M21—The optical rotation of casein (illustrated). D. C. Carpenter, New York Agricultural Experiment Station.
- M22—Studies in rennin coagulation. Factors affecting the nature of the clot. N. P. Tarassuk and G. A. Richardson, University of California.
- M23—The physical changes involved in the rennet coagulation of milk and the subsequent firming of the curd. H. A. Bendixen, State College of Washington.
- M24—Studies in the application of X-rays to research in dairy technology: Structural changes occurring in casein during cheese ripening. S. L. Tuckey, University of Illinois.
- M25—Disturbance of the natural oxidation-reduction equilibrium of milk, with special reference to the use of dehydrated milk in the manufacture of cottage cheese. W. H. E. Reid and R. L. Brock, University of Missouri.
- M26—Methods used to increase blue mold growth in cheese. N. S. Golding, University of Idaho.
- M27—Cream cheese spreads. A. C. Dahlberg and J. C. Marquardt, New York Agricultural Experiment Station.

- M28—Color development in lactose solutions during heating, with special reference to the color of evaporated milk. B. H. Webb, Bureau of Dairy Industry.
- M29—The fluorescence of certain milk constituents in filtered ultra-violet rays. G. C. Supplee and Zaida M. Hanford, The Dry Milk Company.
- M30—Certain foam producing substances of milk. S. Ansbacher, G. E. Flanigan, and G. C. Supplee, The Dry Milk Company.

Thursday morning, June 28, Geneva
Jordan Hall, Room A

ADDRESS OF WELCOME

9:45 A. M.

DIRECTOR U. P. HEDRICK

BUSINESS MEETING

10:00 A. M.

GENERAL SESSION

10:30 A. M.

C. L. ROADHOUSE, *Chairman*

- G7—The iodine content of milk as affected by feeding iodized dry milk. Zaida M. Hanford and G. C. Supplee, The Dry Milk Company; L. T. Wilson, Walker Gordon Laboratories.
- G8—The production of vitamin D evaporated milk by irradiation. K. G. Weckel and H. C. Jackson, University of Wisconsin.
- G9—The commercial production and scientific value of soft curd milk produced by base exchange treatment. H. E. Otting and J. J. Quilligan, M & R Dietetic Laboratories.
- G10—Mineral nutrition (illustrated). R. B. Becker, W. M. Neal, and A. L. Shealy, Florida Agricultural Experiment Station.
- G11—Can nutrition and feeding experiments be improved? F. B. Morrison, Cornell University.
- G12—Reports on the International Dairy Congress and dairy conditions in Europe. R. S. Breed and L. A. Rogers.

Thursday afternoon, June 28; Geneva

(Two sections, Production and Manufacturing, will meet concurrently.)

PRODUCTION SECTION

1:30 P. M.

Jordan Hall, Room A

H. O. HENDERSON, *Chairman*

- P31—The effect of an alfalfa hay ration on the composition of the milkfat. G. A. Richardson, University of California.
- P32—Influence of environmental temperature on the energy metabolism of dairy cows. Max Kleiber, G. A. Richardson, and W. M. Regan, University of California.

- P33—Environmental temperature and the dairy cow. The effect of green pastures. W. M. Regan and G. A. Richardson, University of California.
- P34—Effect of the injection of sterile solutions, milk and oxygen into the udder of the dairy cow on the composition and yield of milk. E. R. Garrison and C. W. Turner, University of Missouri.
- P35—Proven sires and partially proven dams in the Experiment Station herd. A. C. Dahlberg, New York Agricultural Experiment Station.
- P36—The apparent digestibility and feeding value of oat and pea stack silage. J. C. Knott, R. E. Hodgson, and H. K. Murer, State College of Washington and Bureau of Dairy Industry.
- P37—The preparation and nutritive value of A. I. V. alfalfa silage for dairy cows. Departments of Animal Husbandry, Agricultural Chemistry, and Agricultural Bacteriology, University of Wisconsin. (Presented by G. Bohstedt)
- P38—Blood leucocytes in relation to lactation (preliminary report). H. A. Herman and C. W. Turner, University of Missouri.

MANUFACTURING SECTION

1:30 P. M

Jordan Hall, Room B

HAROLD MACY, *Chairman*

- M31—Influence of the period of lactation on leucocyte count of milk. E. O. Anderson, Connecticut State College.
- M32—Some effects of freezing upon milk and cream. B. H. Webb and S. A. Hall, Bureau of Dairy Industry.
- M33—Fat distribution in frozen cream. H. C. Trelogan and W. B. Combs, University of Minnesota.
- M34—A note on storage temperatures of frozen fruits for ice cream. J. C. Hening, New York Agricultural Experiment Station.
- M35—Controlling physical properties of high solids mixes. M. J. Mack, Massachusetts State College.
- M36—The effect of aging temperature on the bacterial count of the ice cream mix. William S. Mueller, Massachusetts State College.
- M37—Coli in milk. J. M. Brannon, University of Illinois.
- M38—Practical limits for bacterial counts in market milk. J. M. Brannon and M. J. Prucha, University of Illinois.

JOURNAL OF DAIRY SCIENCE

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NUMBER 7

THE EFFECT OF HEAT AND CHEMICAL STERILIZATION ON THE RUBBER PARTS OF MILKING MACHINES

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The literature does not cover completely the effect of heat and of the common chemical sterilizing agents on the life (period of usefulness) of the rubber parts of milking machines.⁴ In some milk control areas, the law requires heat sterilization of all dairy equipment, including the rubber parts of milking machines. Because such requirements exist and because there is little information concerning the effect of both heat and chemical sterilization on the life of the rubbers, a detailed study of this subject was included in an investigation of the sanitary operation of milking machines.⁵ The data reported are based on the operation of four units of a milking machine of one manufacturer⁶ which were used for a period of two years in milking the Experiment Station herd.

DETERMINATION OF THE LIFE OF THE RUBBERS

The life of milking machine rubbers depends on several factors, such as quality and age, thoroughness of cleaning, and method of sterilization. In this investigation, factors influencing the life of the rubbers were kept as constant as possible.

Rubber tubes and teat cup liners were assumed to be worn out when they no longer gave efficient service. Air tubes and short milk tubes were discarded when cracked or so stretched at the ends that they would no longer stay on the claws or metal teat cup shells. Teat cup liners were dis-

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⁴ Burgwald, L. H. Cleaning milking machines. U. S. Department of Agriculture Farmers' Bul. 1815. 1923.

⁵ Henderson, J. L., Roadhouse, C. L., and Folger, A. The sanitary care, operation and installation of milking machines. California Agr. Ext. Serv. Circ. 69. November, 1932.

⁶ The De Laval machine was used in these experiments. It is recognized that there is a variation in the effect of heat upon the life of rubber parts for milking machines.

carded when they no longer were elastic enough to draw the milk rapidly or to stay on the cow's teats. Occasionally they became cracked and were discarded. Throughout the experiment the discarding of rubbers was decided upon by the same two persons in order to maintain a uniform standard. Some dairymen might use the teat cup liners longer than the standard established in this investigation, but the rapidity of milking would be retarded thereby.

The life of the long milk tubes depends largely on the number of times they are stepped on by the cows or are trimmed at the ends. With proper care, they lasted approximately one year with both heat and chemical sterilization.

EFFECT OF HEAT STERILIZATION ON LIFE OF RUBBERS

Procedure.—After each operation, the milking machines were rinsed, taken apart, and brushed according to standard procedures.⁵ Once each week the teat cup liners were removed from the metal shells for thorough cleaning and inspection.

The heating was carried out in a rectangular galvanized iron tank, inside which was placed a sloping metal tray, held above the bottom of the tank by metal supports (figure 1). Beneath the tray was a perforated

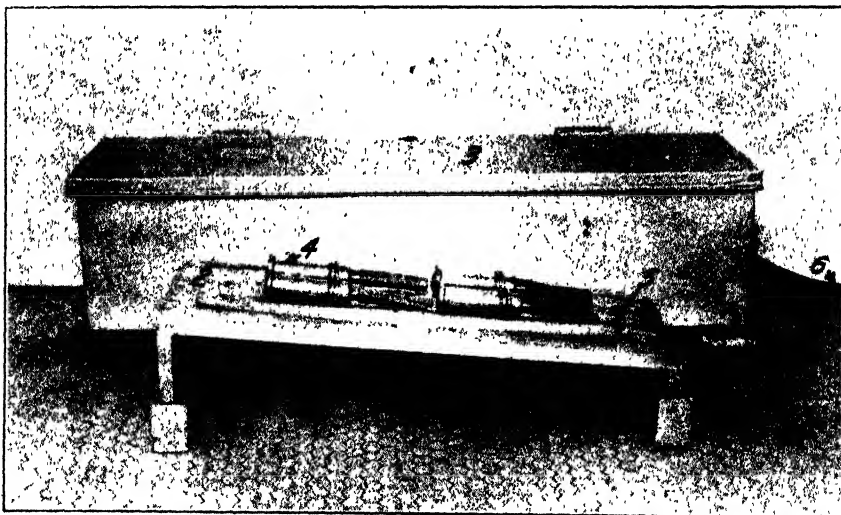


FIG. 1. TANK USED FOR HEAT STERILIZATION.

1. Galvanized iron tank 12×12×50 inches. 2. Corrugated iron tray used to hold rubber parts. The sloping surface prevents air pockets in the long tubes and permits them to drain when the water is drained from the tank. 3. Cover of tank with hole at center for inserting a recording thermometer. 4. Assembled teat cups on tray. 5. Long milk tube on tray. 6. Steam connection to tank. 7. Drain pipe.

steam pipe, used for heating the water. In this tank the rubber parts were covered with sufficient water to maintain the desired temperature throughout the holding period. A metal cover was kept in place to assist in maintaining the temperature and to protect the rubbers from contamination after the water was drawn from the tank following sterilization.

The temperature of heating and the length of time the heat was applied were checked throughout the experiment by a recording thermometer. The heat was applied in water according to the following procedures:

1. Heating to 170° F., holding at that temperature for 20 minutes, and allowing the rubbers to remain in the heated water and to cool gradually until the next milking time.
2. Heating to 170° F., holding at that temperature for 20 minutes, and immediately drawing the water from the tank.
3. Heating to 185° F., and then, without applying further heat, allowing the rubbers to remain in the water for 20 minutes before draining the tank.

TABLE 1

The effect of heat and chlorine sterilization on the rubber parts of milking machines
(Machines were used and sterilized twice daily)

	170° F. FOR 20 MIN. LEFT IN WATER BE TWEEN MILK- INGS	170° F. FOR 20 MIN. WATER THEN DRAINED FROM TANK	185° F.—NO MORE HEAT ADDED. WATER DRAINED FROM TANK AFTER 20 MIN.	CHLORINE 200 P.P.M. IN STERILIZING RACK. FRESH SOLUTION PREPARED DAILY
Teat Cup Liners				
Life in weeks	8	9	8	12
Number of sterilizations	112	126	112	168
Number of cow-hours operated	729	819	729	1,092
Number of sets of rubbers used in determining average life	6	4	6	4
Short Milk Tubes				
Life in weeks	10	19	16	18
Number of sterilizations	140	266	224	252
Number of cow-hours operated	910	1,729	1,456	1,638
Number of sets of rubbers used in determining average life	4	4	2	2
Short Air Tubes				
Life in weeks	20	24	23	25 per cent re- placements after 30 weeks' use
Number of sterilizations	280	336	322	
Number of cow-hours operated	1,820	2,184	2,093	
Number of sets of rubbers used in determining average life	2	2	2	

The effect of these methods of sterilization on the life of the rubber parts is shown in table 1. The machines were operated on an average of 91 cow-hours per unit per week.

Bacterial determinations of the sterile water rinsings of the assembled rubber parts and claws were prepared to check the efficiency of the various sterilization methods. The bacterial counts were satisfactory except when a temperature of 170° F. was used. At this temperature, thermophilic bacteria developed, especially when the rubbers were left in the water until the next milking time.

Results.—The heating of the milking machine rubber parts to 170° F., maintaining that temperature for 20 minutes, and then draining the water from the tank, showed an increased life of the rubber parts as compared with the method of heating and allowing the rubbers to remain in the water until the next milking time. The third method of heating the rubbers (that is, to 185° F.), holding for 20 minutes without further application of heat, and then draining the tank, reduced the life of the rubber parts very slightly when compared with 170° F. for 20 minutes. This method offers the advantages of requiring only one application of heat and of reducing slightly more the bacterial count in the sterile rinse water used to check the efficiency of sterilization. Furthermore, the thermophilic type of organisms was eliminated by this method, as shown in table 3.

Chlorine sterilization of the milking machines used twice a day was also investigated. Table 1 shows that with chlorine sterilization the life of the teat cup liners was 12 weeks; with heat sterilization, only 8 weeks. The strength of the chlorine solution used and its method of application are described under the procedure for chemical sterilization.

EFFECT OF CHEMICAL STERILIZATION ON LIFE OF RUBBERS

Procedure.—In the application of chemical sterilizers, the cleansing of the rubber parts was the same as that described under heat sterilization. The claws, teat cups, and rubber parts were then assembled ready for use and were placed on the sterilizing rack commonly recommended for chemical sterilization (figure 2). The milking machines were operated and sterilized three times daily throughout the tests and were operated on an average of 252 cow-hours per unit per week.

The hypochlorite solution was prepared as follows: one twelve-ounce can of chloride of lime was mixed with one gallon of water, covered, and allowed to stand in an earthen crock for fifteen hours. Then one hundred grams of tri-sodium phosphate was added to the filtered solution to render the solution more stable and to decrease corrosiveness. This stock solution was tested for strength by Fay's method,⁷ and working solutions were prepared each day with water to give a strength of 200 p.p.m.

⁷ Fay, A. C. Preparation, testing, and use of chlorine disinfectants. *Kansas Agr. Exp. Sta. Circ.* 160: 1-8. 1931.

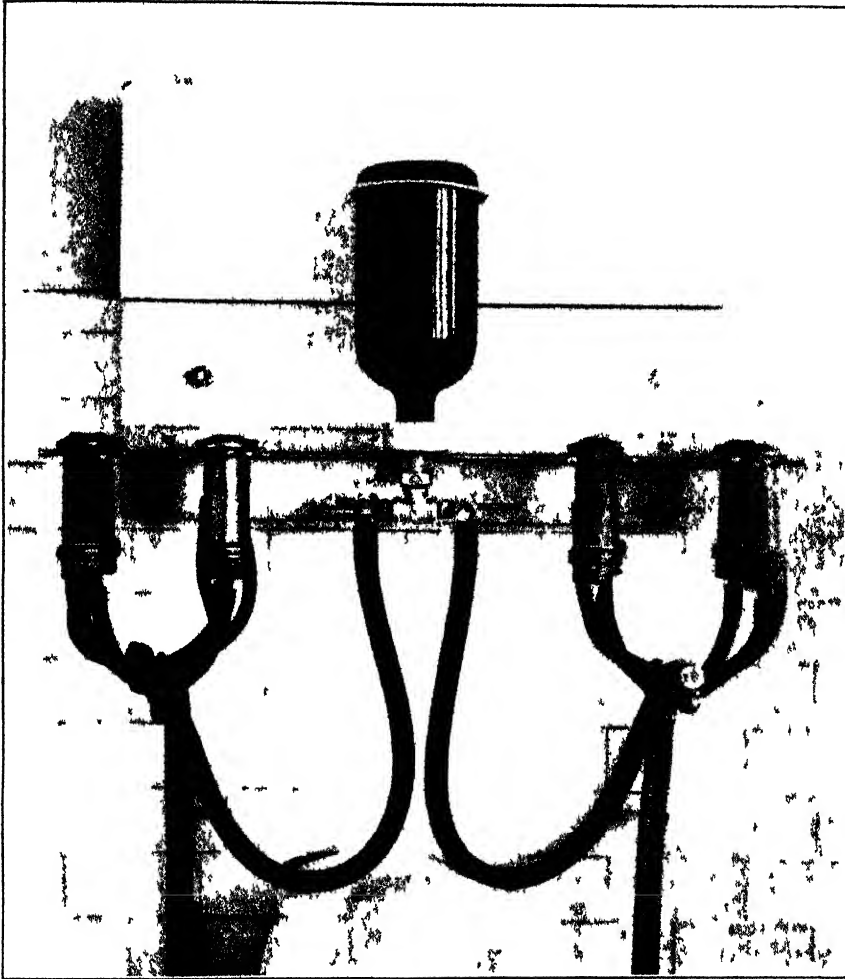


FIG. 2. HOME-MADE CHLORINE OR SODIUM HYDROXIDE STERILIZATION RACK.

A brown glass bottle should be used for chlorine solution in order to protect it from light.

The sodium hydroxide (lye) solutions were prepared by dissolving sodium hydroxide flakes in water to make solutions with strengths of 0.3 and 0.5 per cent. The sterilizer rack used was the standard one recommended for chemical sterilization of rubber parts. The results from the use of chlorine and of sodium hydroxide appear in table 2.

Results.—The treatment of milking machine rubber parts with chlorine of a strength of 200 parts per million, with sodium hydroxide 0.3 per cent solution, each of which was used in the sterilizing rack, and with sodium hydroxide 0.5 per cent solution used in an earthen crock, showed that the 0.3 per cent sodium hydroxide affected the life of rubber parts the same as

TABLE 2

The effect of chlorine and lye sterilization on the life of rubber parts of milking machines
(Machines were used three times per day)

	CHLORINE 200 P.P.M. USED IN STERILIZING RACK (CHANGED DAILY)	NAOH 0.3 PER CENT USED IN STERILIZING RACK (CHANGED DAILY)	NAOH 0.5 PER CENT USED IN EARTHEN CROCK (CHANGED WEEKLY)
Teat cup liners			
Life in weeks	11	11	10
Number of sterilizations	231	231	210
Number of cow-hours operated	2,772	2,772	2,520
Number of sets of rubbers used in determining average life	8	4	4
Short milk tubes			
Life in weeks	14		
Number of sterilizations	294		
Number of cow-hours operated	3,528		
Number of sets of rubbers used in determining average life	6		
Short air tubes			
Life in weeks	31		
Number of sterilizations	651		
Number of cow-hours operated	7,912		
Number of sets of rubbers used in determining average life	2		

did chlorine of 200 parts per million; that is, the teat cup liners gave 11 weeks of service. With the use of sodium hydroxide 0.5 per cent solution, the life of the rubbers was 10 weeks.

The results reported in table 2 were secured from three-times-a-day milkings, and the average life of the tea cup liners totaled 2,772 cow-hours of operation. The use of chlorine, as reported in table 1 in which the teat cup liners lasted 12 weeks, was based on twice-a-day milkings and a total of 1,092 cow-hours of service. Evidently, therefore, the life of the teat cup liners is affected more by the number of weeks of service than by the number of cow-hours of operation. The same results were secured with chlorine sterilization of the short milk tubes and the short air tubes.

EFFECT OF HEAT AND CHEMICAL STERILIZATION ON THE BACTERIA COUNT OF RUBBERS

Procedure.—The bacteria count of the milking machine rubbers was obtained by rinsing the inner surfaces of the rubbers with sterile distilled water and then plating the water. The rinsings were carried out in the

following manner: The teat cups and long milk tubes were attached to the claws, and the assembled units were suspended from the sterilizing rack (figure 2). A sterile cork was inserted in the open end of the long milk tube. One hundred cc. of sterile water was then poured into the teat cups, and the assembled rubbers were shaken in such a way that the water came in contact with all parts of the inner surface of the rubbers. The water removed from the tubes was then plated with standard agar media. In certain instances media made of 15 grams of agar and 20 cc. of skimmilk per liter were used. Incubation of the plates was at 37° C. and 60° C. The higher temperature and the special media were used to detect the presence of thermophilic bacteria.

Results.—The results of the bacteria determinations appear in table 3. They show that of the heat sterilization methods used, the most satisfactory was the procedure of heating to 185° F., allowing the rubbers to remain in the water for 20 minutes, and then draining the water from the tank.

TABLE 3
Bacteria counts of sterile rinse water passed through assembled rubber parts

TREATMENT OF RUBBERS	MEDIA AND INCUBATION TEMPERATURES	AVERAGE NUMBER OF BACTERIA PER CC. OF RINSE WATER	NUMBER OF SAMPLES PLATED
170° F. for 20 minutes left in water between milkings	Heat sterilization		
	Standard 37° C.	125	96
	Standard 60° C.	746	68
	*Special 60° C.	6,430	26
170° F. for 20 minutes. Water then drained from heating tank	Standard 37° C.	11	10
	Standard 60° C.	4,420	10
	Special 60° C.	350	10
185° F. No more heat added, tank drained after 20 minutes	Standard 37° C.	3	14
	Standard 60° C.	0	14
	Special 60° C.	0	14
Chemical sterilization			
Chlorine 200 p.p.m. used in sterilizing rack	Standard 37° C.	45†	114
	Standard 60° C.	0	52
0.3 per cent NaOH solution used in sterilizing rack	Standard 37° C.	372	16
	Standard 60° C.	0	16
0.5 per cent NaOH solution in crock, new solution used once per week	Standard 37° C.	148	18
	Standard 60° C.	0	18

* Special media for determining the presence of thermophiles consisted of 15 grams of agar and 20 cc. of skimmilk per liter.

† Three high counts that occurred in warm weather in July are not included in this average.

Table 1 shows that this method does not shorten the life of the rubbers significantly when compared with heating at 170° F. for 20 minutes. The higher temperature also destroys the thermophilic bacteria, which appear to survive the lower temperature.

When chlorine and sodium hydroxide were used, thermophilic bacteria did not develop, and the total number of bacteria remained within reasonable limits. Chlorine sterilization gave three very high counts during the summer period when it is difficult to maintain the strength of the solution. In the hands of the average dairyman, the use of chlorine is less satisfactory because the rubbers must be free from organic material and the strength of the solution regularly checked, if the germicidal quality is to be assured.

CONCLUSIONS

1. Heat sterilization of milking machine rubber parts, using water at a temperature of 185° F. and leaving the rubbers in the water for 20 minutes, was the most satisfactory method of heat sterilization employed. The life of the rubber parts was not materially reduced by this method when compared with heating at 170° F. for 20 minutes.

2. When the rubbers were heated at a temperature of 170° F. and held at that temperature for 20 minutes, or when they were left in the water to cool gradually until next milking time, there was evidence that thermophilic bacteria developed.

3. Chlorine in the solution of 200 parts per million, or sodium hydroxide solution of a strength of 0.3 and 0.5 per cent, gave bacteria counts somewhat higher than those resulting from heat sterilization at 185° F. They were, however, effective in controlling thermophilic bacteria. The life of the rubbers with chlorine and with sodium hydroxide sterilization was approximately the same.

4. The life of the teat cup liners resulting from each of the different methods of heat sterilization was approximately the same but was 33 per cent shorter than when chlorine of a strength of 200 parts per million was used.

5. In chlorine sterilization, the length of service had more influence in reducing the life of the rubbers than did cow-hours of operation.

IRRADIATED MILK: THE INFLUENCE OF FAT CONTENT AND TIME OF EXPOSURE ON THE ANTIRACHITIC POTENCY

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Studies recently reported (1) show that a large percentage of the incident ultraviolet rays within the antirachitic range are absorbed by milk films less than 0.10 millimeter thick. Notwithstanding this high percentage absorption by the immediate surface layers, a relatively high antirachitic activity was obtained in less than 2 seconds and in some instances in less than 1 second, in rapidly flowing films. The data also revealed that the particular conditions of treatment involving the application of radiations of high intensity to films of known thickness and rate of flow, imparted measurable antirachitic properties to milk derivatives containing little or no butter fat. The degree of activation obtained in milks of variable fat content was not in proportion to the amount of fat present. It has been shown by Hess and coworkers (2) (3) (4) that clinical, as well as the laboratory assay, results from milks containing 1.2 per cent butter fat and 3.6 per cent butter fat were substantially the same when identical methods of irradiation were used. In view of the laboratory and clinical evidence, it has seemed desirable to obtain further information regarding the antirachitic activation as influenced by the character and thickness of the milk film, the fat content and time of exposure.

EXPERIMENTAL

The experimental procedures employed were the same as those already reported, wherein a carbon arc of the same character and intensity of the energy output was used for irradiating milk films on the especially devised flow board which permitted the determination of the film thickness, speed of flow and volume delivered by the film per unit of time (1). The biological data were obtained in the usual manner and the results (Table 1) reduced to the same comparable basis previously used (1) (5) (6).

The results indicate that the maximum antirachitic activity of milk resulting from direct irradiation does not parallel the fat content. It appears, however, that the fat content does influence to a certain but limited degree the rate at which the antirachitic properties are imparted to the milk. Other conditions being the same during the irradiation of films 0.02

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TABLE 1
The Vitamin D concentration of milk as affected by film thickness, film capacity, fat content and amount of applied energy
 (2000-3000 Å²)

SAMPLE	TEST SUBSTANCE	MILK FILM CHARACTERISTICS			EXPOSURE PLATE	TOTAL ENERGY PER CC. (X10 ³)	TOTAL QUANTITY PER CC. (X10 ³)	TOTAL MILK FED	VITAMIN D PER CC. (X10 ¹⁰)	VITAMIN D PER MG OF FAT (X10 ¹⁰)
		Capacity per inch per minute	Thickness	Vertical distance of travel during exposure						
		gms.	mm.	cm.	secs.			cc.	mols.	mols.
1 SM	Skinned Milk	2.12	0.02	0.66	1	8,945	11,851	20	—	—
2 SM	"	"	"	10.79	16	143,120	179,610	50	—	300.0
3 SM	"	"	"	21.61	32	286,240	359,220	40	—	373.0
4 SM	"	"	"	48.64	72	644,060	853,272	40	—	373.0
1-06	Milk, 0.6% Fat	2.12	0.02	0.66	1	8,945	11,851	60	—	12.5
2-06	"	"	"	5.38	8	71,560	89,805	30	—	25.0
3-06	"	"	"	10.79	16	143,120	179,610	30	—	25.0
4-06	"	"	"	48.64	72	644,060	853,272	30	—	25.0
5-06	"	34.02	0.11	40.64	1.87	3,769	4,994	60	—	12.5
1-12	Milk, 1.2% Fat	2.12	0.02	0.66	1	8,945	11,851	60	—	6.25
2-12	"	"	"	5.38	8	71,560	89,805	30	—	12.50
3-12	"	"	"	10.79	16	143,120	179,610	20	—	18.75
4-12	"	"	"	48.64	72	644,060	853,272	20	—	18.75
5-12	"	34.02	0.11	40.64	1.87	3,769	4,994	20	—	18.75
6-12	"	192.78	0.23	40.64	0.89	972	1,288	50	—	7.50
1-36	Milk, 3.6% Fat	2.12	0.02	0.66	1	8,945	11,851	30	—	4.16
2-36	"	"	"	5.38	8	71,560	89,805	15	—	8.32
3-36	"	"	"	10.79	16	143,120	179,610	15	—	8.32
4-36	"	"	"	48.64	72	644,060	853,272	15	—	8.32
5-36	"	8.78	0.10	5.08	1	2,236	2,692	20	—	6.25
6-36	"	"	"	9.52	1.87	4,181	5,538	15	—	8.32
7-36	"	"	"	40.64	8	17,888	23,696	15	—	8.32

TABLE 1—(Continued)
The Vitamin D concentration of milk as affected by film thickness, film capacity, fat content and amount of applied energy
 (2000–3000 Å°)

SAMPLE	TEST SUBSTANCE	MILK FILM CHARACTERISTICS			EXPOSURE PERIOD	TOTAL CC. (X10 ³)	TOTAL QUANTA PER CC. (X10 ¹¹)	TOTAL MILK FED cc.	VITAMIN D PER CC. (X10 ⁶)	VITAMIN D PER MO. OF FAT (X10 ¹⁰)
		Capacity per inch per minute	Thickness	Vertical distance of travel during exposure						
8-36	Milk, 3.6% Fat	gms. 34.02	gms. 0.11	cm. 21.60	secs. 1	2,016	2,671	30	moles. 150	4.16
9-36	" "	" "	" "	40.64	1.87	3,769	4,994	20	225	6.25
10-36	" "	" "	0.23	33.02	1	976	1,293	35	128	3.55
11-36	" "	102.06	" "	40.64	1.24	1,210	1,603	25	180	5.00
12-36	" "	" "	" "	61.72	1.87	1,825	2,418	15	300	8.32
13-36	" "	192.78	0.23	40.64	0.89	972	1,288	35	128	3.55
14-36	" "	" "	" "	76.20	1.87	2,041	2,704	25	180	5.00
15-36	" "	272.16	0.46	40.64	0.89	486	644	37	121	3.36
16-36	" "	" "	" "	76.20	1.87	1,020	1,352	30	150	4.16
17-36	" "	442.26	0.50	40.64	0.60	447	592	40	112	3.11
18-36	" "	" "	" "	66.80	1	742	989	40	112	3.11
19-36	" "	" "	" "	125.00	1.87	1,394	1,847	25	180	5.00
1-72	Milk, 7.2% Fat	2.12	0.02	0.66	1	8,945	11,851	17.5	257	3.56
2-72	" "	" "	" "	5.38	8	71,560	89,805	17.5	257	3.56
3-72	" "	" "	" "	10.79	16	143,120	179,610	17.5	257	3.56
4-72	" "	" "	" "	48.64	72	644,060	179,610	17.5	257	3.56
5-72	" "	34.02	0.11	40.64	1.87	3,769	4,994	17.5	257	3.56

millimeter thick a higher antirachitic potency is produced in milks containing 3.6 per cent fat and 7.2 per cent fat during the first momentary exposure (about 1 second) than is produced in milks of lower fat content. When the milks of low fat content irradiated in the thinnest obtainable films are subjected to longer exposure periods, the antirachitic property is increased during a period of about 16 seconds; the period during which there is an increase being the longest for the milk containing the least amount of fat. The period during which there is an increase in the potency of milk containing 3.6 per cent fat (whole milk) appeared to be limited to about 8 seconds, under similar conditions of treatment. When milk containing 7.2 per cent butter fat is similarly treated there appears to be no increase in potency after the first second of exposure. The period during which the antirachitic potency of whole milk develops most rapidly when irradiated in thicker and more rapidly flowing films, appears not to exceed about 2 seconds. This seems to again confirm the conclusion that vitamin D synthesis in milk during direct irradiation is substantially instantaneous in the immediate surface layer. The homogenization of whole milk prior to irradiation, whereby the surface area of a unit amount of fat exposed to the milk serum is greatly increased, does not permit any significantly greater degree of activation, or increased rate of activation, under the conditions used, than does the unhomogenized milk.

The data presented in this paper clearly indicate that substances contained in milk other than the milk fat are important factors which determine its antirachitic properties during irradiation. It is quite probable that a further investigation of such substances will explain wholly, or in part, the merits of irradiated milk as shown by the clinical investigations of Hess and Lewis (3) (4), and others. The rapidity of vitamin D synthesis during momentary exposure periods and the failure to induce a further significant increase in potency during longer exposure periods, raises certain questions regarding the mechanics of activation. A full explanation of the results may necessitate the consideration of many factors concerning the substance or substances affected by radiant energy. Notwithstanding the possible influence of the chemical constitution and physical relationship of such substances as they exist in milk, the observations of Bourdillon *et al.* (7), Reerink and Van Wijk (8), Marshall and Knudson (9) and Windaus *et al.* (10), are, no doubt, pertinent, in certain respects at least, to the results recorded herein.

CONCLUSIONS

1. The fat content influences to a certain but limited degree the rate at which antirachitic properties are produced in milk by direct irradiation with ultraviolet rays.

2. Milks containing little or no butter fat may be activated to a substan-

tial degree, but the degree of potency ultimately obtained is not reached as quickly as in those milks containing larger amounts of fat. The degree of potency attainable in milk with the larger amounts of fat does not increase in proportion to the amount of fat present.

3. Milks containing normal amounts of fat may be activated substantially to their maximum degree by a momentary exposure of less than 2 seconds, if suitable intensity of ultraviolet radiations are applied to films of suitable thickness and flow characteristics.

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REFERENCES

- (1) SUPPLEE, G. C., AND DORCAS, M. J. Irradiated milk: The transmission and antirachitic activation of milk films by ultra-violet radiations. *JOUR. DAIRY SCIENCE* 17: 433. 1934.
- (2) SUPPLEE, G. C., DORCAS, M. J., AND HESS, A. F. Irradiated milk: The energy requirements for antirachitic activation. *Jour. Biol. Chem.* 94: 749. 1932.
- (3) HESS, A. F., AND LEWIS, J. M. Milk irradiated by the carbon arc lamp: A clinical and laboratory study of rickets. *Jour. Am. Med. Assoc.* 99: 647. 1932.
- (4) HESS, A. F., AND LEWIS, J. M. An appraisal of antirachitics in terms of rat and clinical units. *Jour. Am. Med. Assoc.* 101: 181. 1933.
- (5) SUPPLEE, G. C., HANFORD, Z. M., DORCAS, M. J., AND BECK, H. H. Irradiated milk: The amount of vitamin D and its rate of formation. *Jour. Biol. Chem.* 95: 687. 1932.
- (6) SUPPLEE, G. C., BECK, H. H., AND DORCAS, M. J. Irradiated milk: The influence of the intensity and character of the radiations on the antirachitic potency. *Jour. Biol. Chem.* 98: 769. 1932.
- (7) BOURDILLON, R. B., FISCHMANN, C., JENKINS, R. G. C., AND WEBSTER, T. A. Absorption spectrum of vitamin D. *Proc. Roy. Soc. London, Series B*, 104: 561. 1929.
- (8) REERINK, E. H., AND VAN WIJK, A. The vitamin D problem: The photochemical reactions of ergosterol. *Biochem. Jour.* 23: 1294. 1929.
- (9) MARSHALL, A. L., AND KNUDSON, A. The formation of vitamin D by monochromatic light. *Jour. Am. Chem. Soc.* 52: 2304. 1930.
- (10) WINDAUS, A., AND LUTTREINGHAUS, A. Uber das bestrahlte Ergosterin. *Deut. Med. Woch.* 58: 1669. 1932.

THE EFFECT OF PASTEURIZATION UPON THE VITAMIN C CONTENT OF MILK

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The protection from bacterial infections which is afforded by pasteurization of municipal milk supplies is an acknowledged factor of great importance. In general those who are actively working in the field of dairy science, particularly in relation to public health, are agreed that the advantages of proper pasteurization greatly outweigh the possible minor disadvantages (1). The further elimination of any apparent disadvantages represents important progress insofar as it tends (a) to increase the practice of proper pasteurization, or, (b) to improve the quality and quantity of milk which is consumed.

Of the recognized nutritive factors in milk, vitamin C is probably the most sensitive to destruction, due primarily to its oxidation. Such oxidative changes are greatly accelerated by exposure to air at high temperatures and by the presence of metallic catalysts, particularly copper (2) (5). Hence a quantitative study of the effect of pasteurization upon the vitamin C content of milk should provide an extremely sensitive index to possible changes in its chemical nature or its nutritive value.

The present investigation has included a quantitative study of the vitamin C content of representative western Pennsylvania market milk as received at the pasteurizing plant, and the effect of pasteurization by the (a) Electropure, (b) StamVik, and (c) holding processes, each of the three units receiving milk from the same agitated raw milk tank. The primary purpose of the investigation was to find whether there was a significant amount of vitamin C destroyed during the Electropure or StamVik processes of pasteurization.

The dairy herds supplying milk were chiefly on summer pasture, supplemented with varying amounts of hay and grain.

PASTEURIZING EQUIPMENT

The Electropure pasteurizer (3) (Figure 1) was a small standard commercial unit (capacity 100 gal. per hour), equipped throughout with aluminum piping and fittings. The upper part of the cooler served as a heat exchanger, preheating the milk to approximately 125° F., after which it passed through a filter and thence to the Electropure heating unit where the temperature was raised to 161–3° F. by the resistance of the upward-flowing column of milk (between carbon electrodes, 220 V. A.C.). Having

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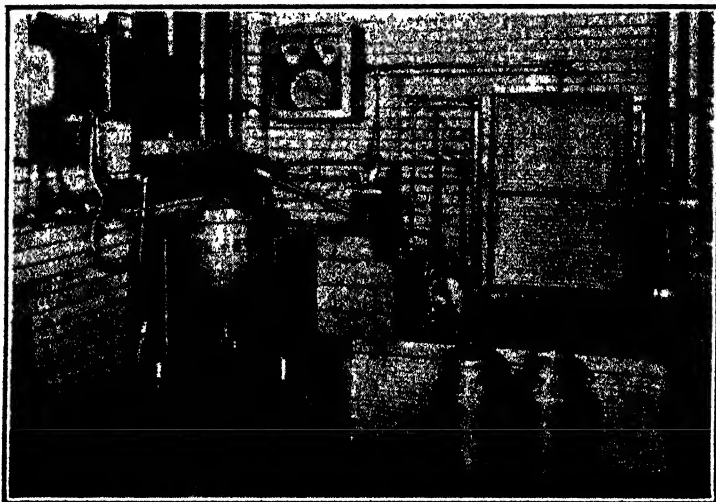


FIG. 1

reached the pasteurizing temperature, there was a time interval of 16 seconds before the milk reached the top of the cooler, the rate of flow being controlled automatically by the speed of a pump which was in turn regulated by the temperature of the milk leaving the heating chamber. The lower section of the cooler was supplied with brine to complete the cooling.

The StamVik pasteurizer (Figure 2) was a standard commercial unit (4) (capacity 500 gal. per hour), also equipped throughout with aluminum piping and fittings. The cooler-regenerator and filter equipment was of the same type as that in the Electropure unit (entirely aluminum). The

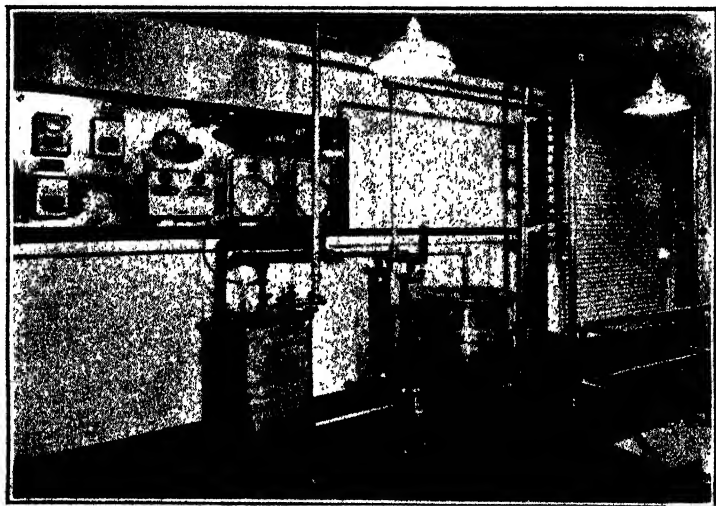


FIG. 2

essential characteristic of the StamVik process is the heating unit, constructed of a series of hollow aluminum plates, with counter current heating of the milk by means of hot water flowing inside the aluminum plates (4).

The time interval for reaching the pasteurizing temperature in the heating unit (161–3° F.) is only 17 seconds, in which respect it approaches the Electropure process, where the corresponding interval is approximately 4 seconds for the unit used.

During the greater part of the experimental period, pasteurization by the holding method was carried out in laboratory-scale equipment. During the latter part of the study a commercial pasteurizing vat became available (150 gal. capacity), which afforded an opportunity of verifying the results obtained on a laboratory scale. Raw milk was drawn from the same tank which supplied the other units into a new, heavily-tinned covered milk can which was placed in a large vat of hot water adjusted to raise the milk to pasteurizing temperature (143–145° F.) in five to ten minutes, with slow agitation. As soon as 142° F. was reached, the vat of water was cooled to 143–5° and held at that temperature for 30 minutes. The milk was then cooled quickly over a brine cooler (identical with that used for the milk from the other processes) from which bottles were filled and capped and placed in refrigeration (40–50° F.) until used.

During the latter part of the period, normal plant practice was followed in pasteurizing for 30 minutes in a commercial covered, tin-lined vat at 143–5° F. with normal agitation during heating by a rotating hot water coil, after which agitation was stopped until the pasteurization was completed. It was then agitated slowly as it flowed to the cooler, the bottled samples being taken from the bottom of the cooler after about five minutes.

Samples of raw milk were taken from the same supply used for pasteurizing by the three different methods, and all bottled samples (3 of each per day) were placed in a crate together for refrigeration until delivered to the laboratory the same day or the following morning.

Plant operations were carried out by workmen in the dairy who made every effort to keep the products as nearly comparable in every way as was possible under plant conditions.

ANIMAL ASSAYS

Young male guinea-pigs were purchased whose average weight was about 300 grams. They were placed in individual cages and fed a modified Sherman diet (supplemented by cod-liver oil) as a basal vitamin C-free ration. The only modification in the basal ration was to decrease the amount of milk solids equivalent to 30 cc. of fresh whole milk per day, to compensate for the additional supply of milk in test feeding. During a preliminary period of 7 to 10 days, the animals were fed a supplement of fresh spinach and young, crisp carrots, to assure that they were growing

normally, in apparent good health, and in a good state of nutrition at the beginning of the test period. Each animal was given 10 cc. of milk for two days before the test period began, to accustom it to drinking from a pipette.

From the beginning of the assay period, the animals received only their basal diet and water, supplemented by the 30 cc. of milk being tested, fed directly from a pipette. The results of the test are given in table 1.

TABLE 1
Feeding test with 30 cc. of milk as the only antiscorbutic

TYPE OF MILK FED	NO. OF ANIMALS	WEIGHT IN GRAMS	NO. ANIMALS SURVIVING 56-DAY TEST	AVER. SURVIVAL OF THOSE DYING BEFORE 56 DAYS	AVERAGE SCURVY SCORE AT AUTOPSY
Raw	8	327	3	40	7.1
Vat Pasteurized	8	325	0	27	14.8
Electropure " ..	8	332	2	44	7.0
StamVik " .	8	327	1	42	8.0

Realizing that general market milk would be somewhat low in vitamin C value, we were reasonably sure that 30 cc. per day would not be adequate for complete protection (5). Feeding larger quantities, up to the amount required for full protection, *e.g.*, 60 to 80 cc., involves keeping the animals on practically a whole-milk diet, which is not suitable for animals accustomed to a diet high in roughage. Hence we decided to use one series of animals receiving only the 30 cc. of milk supplement, and another receiving milk and an additional supplement of orange juice. The former furnished a basis for direct comparison based upon survival and weight change, and the latter served to raise the total vitamin intake to a level which permitted better survival and growth, and consequently a more satisfactory basis for evaluating small differences. Each group received exactly the same supplement of orange juice.

The animals receiving orange juice were fed carefully from graduated pipettes, so that all received exactly the same amount—the quantity being gauged to keep most of the animals from losing weight or developing severe scurvy. The milk supplement was raised from 15 to 30 cc. for these groups on the 28th day. In earlier studies with the pure vitamin (6) it has been found that a daily intake of 0.5 mg. per day provides an excellent level for quantitative interpretation. Approximately such a level was provided by feeding 15 to 30 cc. of milk and 0.5 to 1.0 cc. of orange juice daily. For assaying and comparing foods which are relatively low in vitamin C, we believe such a procedure has a distinct advantage, in that it (a) permits the animals to receive a more nearly normal dietary, (b) supplies a vitamin level most sensitive to assay differences, and (c) provides a marked economy in feeding time. Table 2 furnishes a summary of the results obtained.

TABLE 2
Feeding test, milk supplemented by 1 to 2 cc. of orange juice

TYPE OF MILK FED	NO. OF ANIMALS	WEIGHT IN GRAMS	NO. ANIMALS SURVIVING 56-DAY TEST	AVER. SUR- VIVAL OF THOSE DYING BEFORE 56 DAYS	AVERAGE SCURVY SCORE AT AUTOPSY
Raw	7	290	2	37	1.8
Vat Pasteurized ..	7	296	7		7.0
Electropure " ..	7	314	7		1.4
StamVik " ..	7	317	5		1.8

TITRATION OF VITAMIN C

The oxidation-reduction indicator, 2, 6-dichlorophenolindophenol, introduced by Mansfield Clark and associates (7), was first adapted for vitamin C titration by Tillmans and associates (8). With reasonable reservation in considering possible interfering substances, we have found the method to be applicable for studying most plant and animal tissues. Details to be followed in using the indicator and data obtained by its use have been given in another paper (9). The titration of vitamin C in milk proved to be relatively satisfactory in the presence of trichloroacetic acid and furnished data in good agreement with the animal feeding tests, as shown by the summary of the titrations given in table 3.

TABLE 3
Direct titration of vitamin C in milk

TYPE OF MILK	APPROXIMATE TITRATION VALUE MG. VITAMIN PER 10 CC.
Raw	0.10
Vat Pasteurized	0.04
Electropure Pasteurized	0.10
StamVik Pasteurized	0.10

DISCUSSION OF RESULTS

It has been shown in previous investigations (3) (5) that prolonged heating of milk exposed to air tends to destroy vitamin C more severely than heating to the boiling point for a short time. In the Electropure and StamVik processes there is only a very short interval for preheating and pasteurizing (16 seconds after reaching 162° F.) during which time the milk is protected from air. The time of exposure as the milk flows over the surface cooler is approximately 6 seconds. This, together with the use of aluminum equipment probably accounts for the finding that there was no significant loss in vitamin C as a result of pasteurization by either process.

The same conclusion is even more definitely indicated by the direct chemical titration data.

Although the animal assay of milk pasteurized by the holding method included that from a commercial unit and in part that from a laboratory unit, there was no evident change in the animal response when the type of pasteurizer was changed, and it is believed that there was no significant difference in the results with laboratory and commercial equipment. Frequent titrations of the two products with the dye-indicator would have demonstrated even smaller differences that could be detected by animal assays. A further study of vitamin C destruction by other pasteurization methods and with different types of equipment will be reported in the near future.

The degree of protection from scurvy afforded by 30 cc. of raw milk is in good agreement with previous data on the vitamin content of cow's milk and also in good agreement with the data obtained by direct chemical titration.

The destruction of a significant portion of the vitamin C content by slow pasteurization is accounted for by the prolonged opportunity for oxidation, resulting in part from exposure to air and perhaps in part by a greater exposure to metals, especially where small quantities of copper may be picked up from well-tinned commercial equipment such as used in the present study.

In view of the relatively rapid depletion of vitamin C from the body tissues when the diet is deficient, and in view of the fact that considerable numbers of children and infants receive only haphazard supplies of supplementary antiscorbutic foods when on a high-milk diet, it is an item of importance to conserve the vitamin content naturally provided. Unfortunately, a large portion of our population does not yet realize the need for providing special antiscorbutic foods to very young children deprived of mother's milk. Although clinical survey is not considered common in America, it does occur occasionally, and there is good evidence to indicate that vitamin deficiencies are significant in relation to health at levels far above those characterized by the so-called deficiency diseases (5) (9) (10). Dalldorf has reported finding an incidence of "latent scurvy" revealed by weakened blood capillaries, reaching 35 to 66 per cent of the children in poor families in New York (11).

The poorer survival on an equal dietary level shown by the animals receiving raw milk was apparently due entirely to the higher incidence of infections in this group. The experimental work was carried out during July and August when the bacterial counts were relatively high. The animals receiving pasteurized milk had an advantage in being protected from infectious types of organisms almost certain to be present in a general raw milk supply. Such biological factors serve to emphasize the value of

knowing the chemical nature of the vitamins and of having available chemical methods of estimation. The scurvy scores of the animals furnish a fairly specific indication of their nutritive condition, however, so that the above interpretation of vitamin C intake on the different types of milk was not misinterpreted by variations in survival alone.

The authors believe that the above experimental record gives strong evidence against the suggestion occasionally made that pasteurization necessarily brings about a significant impairment in the nutritive value of milk. It is unlikely that there is any essential nutritive factor present which is less stable than vitamin C, but there is constantly accumulating evidence for the need of protection from infections (12).

SUMMARY

There is no significant destruction of vitamin C in milk by the Electropure (electrical conductivity) or StamVik (flash contact) methods of pasteurization when all-aluminum equipment is used. This is probably due to: (a) a very short heating time, (b) methods of heating, (c) protection from atmospheric oxidation during heating, and (d) a minimum exposure to metals which catalyze oxidation.

The finding is based upon two series of animal assays of the raw and pasteurized milk and also upon titration of the vitamin by means of 2, 6-dichlorophenolindophenol.

Pasteurization of milk from the same tank either on a laboratory scale or in a commercial vat at 143–5° for 30 min. resulted in a significant destruction of vitamin C.

The importance of conserving the natural vitamin C content of milk during pasteurization has been indicated.

REFERENCES

- (1) PARK, W. H., AND HOLT, L. E. *Med. News* 83: 1066. 1903.
ZINSSER, H., AND RUSSELL, F. F. *Bacteriology*, Chap. 53. (D. Appleton & Co., N. Y.). 1925.
ROGERS, L. A. *The fundamentals of dairy science*, Chap. 10 and 14. (Chemical Cat. Co., N. Y.). 1928.
HESS, A. F., AND UNGER, L. J. *Am. J. Dis. Children* 17: 221. 1919.
- (2) HESS, A. F., AND WEINSTOCK, M. *J. Am. Med. Assoc.* 82: 952. 1924.
ANDERSON, E., DUTCHER, R. A., ECKLES, C. H., AND WILBUR, J. W. *Science* 53: 446. 1921.
SCHWARTZ, E. W., MURPHY, F. J., AND COX, G. J. *J. Nutrition* 4: 211. 1931.
- (3) KING, C. G. *Trans. Am. Electrochem. Soc.* 62: 233. 1932.
- (4) STAMSVIK, A. U. S. Pat. No. 1,916,395 (July 4, 1922) and No. 1,918,434 (July 18, 1933).
- (5) SHERMAN, H. C., AND SMITH, S. L. *The vitamins*, Chap. 4. (Chem. Cat. Co., N. Y.). 1931.
SHERMAN, H. C. *Am. J. Public Health* 23: 1031. 1933.

- (6) WAUGH, W. A., AND KING, C. G. *Science* 75: 357, 360. 1932; *J. Biol. Chem.* 97: 325. 1932.
WAUGH, W. A., BESSEY, O. A., AND KING, C. G. *Trans. Am. Soc. Exptl. Biol. Med.* 30: 1281. 1933.
- (7) COHEN, B., GIBBS, H. D., AND CLARK, W. M. *Pub. Health. Rep., U. S. P. H. S.*, 39: 804. 1924; 40: 649. 1925.
- (8) TILLMANN, J., HIRSCH, P., AND HIRSCH, W. *Z. Untersuch. Lebensmittel* 63: 1. 1932.
- (9) BESSEY, O. A., AND KING, C. G. *J. Biol. Chem.* 103: 687. 1933.
- (10) SHERMAN, H. C. *Chemistry of food and nutrition*, Chap. 24. (Macmillan Co., N. Y.). 1932.
SURE, B. *The Vitamins*, Chap. 4 and 9. (Williams and Wilkins, Balt.). 1933.
MCCOLLUM, E. V., AND SIMMONDS, N. *The newer knowledge of nutrition*, p. 506. (Macmillan Co., N. Y.). 1929.
- (11) DALLDORF, G. *Am. J. Dis. Children* 46: 794. 1933.
- (12) BIGELOW, G. H., AND FEEMSTER, R. F. *Am. J. Pub. Health* 23: 571. 1933.
BIGELOW, G. H., AND FORSBECK, F. C. *Am. J. Pub. Health* 23: 1094. 1933.
BROOKS, P. B. *Am. J. Pub. Health* 23: 1165. 1933.
HUCKER, G. J. *N. Y. Agr. Expt. Sta., Tech Bul. No. 199.* 1933.

RATE OF GROWTH AND ACID PRODUCTION OF *STREPTOCOCCUS LACTIS*

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Evidence has been published elsewhere which indicates that slow growth enables an organism to better adapt itself to its environment and hence to exhibit greater viability when exposed to deleterious environmental factors. It has also been pointed out that among bacteria and higher organisms those species which have become adapted to growth under conditions which are unfavorable for most forms of life have relatively slow rates of growth.¹

If this hypothesis is correct, it is logical to expect that with any given organism the more slowly growing strains should show greater tolerance for the environmental impediments with which it has had to contend during its course of evolution. In the case of *Streptococcus lactis*, acidity is the most obvious limitation to growth under the only natural conditions we know it—in milk and milk products. With this thought in mind, we have attempted to learn whether or not there does exist a relationship between rate of growth and total acid producing power (acid tolerance) in *Streptococcus lactis*.

While we do not wish to draw sweeping conclusions from the small body of data contained in this note, it is presented for what it may be worth as a contribution to the general problem and, also, because it is barely possible that this line of approach might yield useful results in the selection and development of strains of *Streptococcus lactis* for certain dairy purposes.

An obvious way to test the theory would be to isolate a number of cultures of *Streptococcus lactis* and determine the rate of growth and acid producing power of each. This procedure was not considered sound, and was not attempted, because in a given pure culture there may be slow and rapidly growing strains, one of which would give the culture the characteristic of fast growth and the other that of acid tolerance. (However, it may be mentioned incidentally that of the two cultures used in this study the one which grew more slowly had the ability to produce the greater amount of acid.) It is apparent that in any given culture, unless very old, the fastest growing strains would be present in greatest numbers, while the strains which have a slower rate of reproduction would naturally occur in smaller numbers. With this fact in mind it was reasoned that, if our working hypothesis is correct, two things should be demonstratable:

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¹ Sherman, J. M., and Cameron, G. M. Jour. Bact. 27: 23. (Abstract. Data in full not yet published.) 1934.

(1) If a series of flasks of sterile milk were inoculated from a milk culture of *Streptococcus lactis* in amounts ranging from say 1 cc. to 10^{-8} cc., a greater amount of acid should be produced in those receiving the larger inocula. The reason for expecting this result is that in the high dilutions of the original culture there would be present only the most rapidly growing strains which, according to the hypothesis under test, could not endure such a high degree of acidity.

(2) If a culture of *Streptococcus lactis* were grown in milk with very frequent transfer for a period of time, the resulting culture should have a lower acid producing power than the original culture; and, further, contrary to the above case, the amounts of acid produced from varying amounts of inocula should be more nearly the same. The reason for expecting these results is simply that with frequent transfers the slow growing strains would be "weeded out" and left behind so that the resulting culture would contain only fast growing organisms.

A laboratory stock culture of *Streptococcus lactis* (No. 8) 24 hours old in litmus milk was inoculated into flasks of sterile skimmed milk, the inocula varying in amount from 1 cc. to 10^{-8} cc. These flasks, sealed with sterile rubber stoppers, were incubated at 37° C. for 7 days and then titrated for total acidity. Quadruplicate flasks were inoculated with each dilution. The original culture was then carried in litmus milk at laboratory temperature with 12-hour subculturing for ten transfers; this being done in test tubes containing about ten cc. of milk and transfers made with an ordinary inoculating loop. At the end of this period, flasks of sterile skimmed milk were inoculated with this culture for acid production as in the case of the parent culture. The results obtained are given in table 1.

An inspection of these data shows that the predicted results were obtained: (1) The amount of acid produced by the original culture decreased with the size of the inoculum. (2) A similar variation in acid production

TABLE 1
Acid Production by Varying Amounts of Streptococcus lactis (No. 8)

INOCULUM (CC.)	PER CENT ACID AS LACTIC ACID	
	24-hour Stock Culture	Serial 12-hour Culture
1	0.82	0.71
10^{-1}	0.76	0.71
10^{-2}	0.75	0.70
10^{-3}	0.76	0.69
10^{-4}	0.75	0.68
10^{-5}	0.76	0.70
10^{-6}	0.76	0.69
10^{-7}	0.78	0.68
10^{-8}	0.72	No growth

with size of inocula did not occur in the serial 12-hour culture; nor was the amount of acid produced from the 1 cc. inoculum as great as in the case of the original culture.

The experiment was repeated using another strain of *Streptococcus lactis* (No. 21) with similar results (table 2).

TABLE 2
Acid Production by Varying Amounts of Streptococcus lactis (No. 21)

INOCULUM (CC.)	PER CENT ACID AS LACTIC ACID	
	24-hour Stock Culture	Serial 12-hour Culture
1	0.71	0.63
10 ⁻¹	0.67	0.62
10 ⁻²	0.65	0.62
10 ⁻³	0.65	0.62
10 ⁻⁴	0.65	0.61
10 ⁻⁵	0.64	0.62
10 ⁻⁶	0.65	0.63
10 ⁻⁷	0.65	0.62
10 ⁻⁸	No growth	0.62

It is of course possible that the decreasing amounts of acid produced with decreasing amounts of culture is due to some unrecognized factor and not to a negative correlation between rapidity of growth and acid tolerance. If such is the case, it is still difficult to explain why the same phenomenon is not found in the serial 12-hour culture; and also why a smaller amount of acid is produced from a one cc. amount of this culture than from a similar amount of the parent culture.

One point should be mentioned, however, which made us somewhat skeptical about our interpretation of these facts—the marked decrease in amount of acid produced from an inoculum of 0.1 cc. as compared with 1 cc. of the original cultures. Though mathematically possible, it is difficult to believe that organisms which are present in numbers of less than ten per cc. in a 24-hour culture could increase sufficiently within seven days to have a measurable effect upon the final acidity. On the other hand, it is entirely possible that when the initial seeding of the slowly growing strains is very small they would be inhibited by the rapidly growing organisms, as was shown by Rogers² in a study of the inhibition of *Lactobacillus bulgaricus* by *Streptococcus lactis*.

In order to settle that point and test further our interpretation of these results another experiment was performed: Instead of using a 24-hour stock culture, it was tested after incubation for seven days at 37° C. If the higher acidity is produced by slower growing strains, there should be no

² Rogers, L. A. Jour. Bact. 16: 321. 1928.

difference in the amounts of acid produced from differing amounts of the culture under these conditions, as the slowly growing organisms should be present in large numbers. The results of this experiment with the two stock cultures are given in table 3.

TABLE 3
Acid Production by Varying Amounts of Aged Cultures of Streptococcus lactis

INOCULUM (cc.)	PER CENT ACID AS LACTIC ACID	
	Culture No. 8	Culture No. 21
1	0.79	0.77
10 ⁻¹	0.79	0.77
10 ⁻²	0.79	0.77
10 ⁻³	0.79	0.77
10 ⁻⁴	0.79	0.77
10 ⁻⁵	0.78	No growth
10 ⁻⁶	0.79	No growth
10 ⁻⁷	No growth	No growth

It will be noted from these data that in the case of a seven days' old culture, varying the amount of the inoculum did not change the final degree of acidity produced. This would appear to add distinct weight to the hypothesis that slow growth is associated with greater acid producing power.

A word of explanation should be added: This conclusion does not mean that there will be a rigid correlation between growth rate and acid production when various cultures of *Streptococcus lactis* are compared with each other. It is probable that many strains could be found which have slow growth rates and feeble acid producing power. However important the rate of growth may be, it is likely to prove of less significance than inheritance, even among bacteria.

SUMMARY

By segregating the fast growing strains from those which grow more slowly in stock cultures of *Streptococcus lactis*, it has been shown that slow growth is associated with greater acid tolerance.

The conclusion is drawn that such a relationship exists among strains of the same genetic constitution.

SOME PHYSICO-CHEMICAL PROPERTIES OF LACTOSE

I. THE SPONTANEOUS CRYSTALLIZATION OF SUPER- SATURATED SOLUTIONS OF LACTOSE

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During the past few years, research has been under way in the dairy chemistry laboratory at Cornell University directed toward the commercial production of beta lactose, an optical isomer of ordinary lactose. While working on that problem, the need for more data regarding the physical properties of lactose became evident. A search of the literature revealed that information on some subjects was entirely lacking; on others, the data were discordant; and in a few cases, the data found were suspected of error.

For these reasons, a series of researches were undertaken with the purpose of searching for errors in the literature, and of gathering new data which might serve as a basis for future research on lactose. The results of some of these investigations are reported in this series of papers on the physico-chemical properties of lactose.

No attempt will be made to give a complete review of the literature dealing with lactose. Such a review has already been published by Whittier (30). However, references to papers on special topics will be given as they are needed.

Before discussing the experimental work, it seems desirable to describe, briefly, the various modifications of lactose, and the terminology used to describe them. Lactose, or milk sugar, is a disaccharide having a free aldehyde group. Accordingly, two isomeric forms are known. The form having the higher optical rotation is known as alpha lactose; the form having the lower rotation is termed beta lactose. In aqueous solutions, an equilibrium is established between these two forms. The relative amounts of the two sugars present at equilibrium varies with the temperature. This mixture is referred to as equilibrium lactose, or as equilibrium mixture.

The lactose of commerce is a hydrate. In Hudson's early papers, this hydrate was regarded as having two hydroxyl groups on the terminal carbon atom. It was, therefore, related equally to both the alpha and beta forms. This view was opposed by Lowry (15) and later by Gillis (6), and it has apparently been abandoned by Hudson (10). In these papers, the hydrate of lactose is regarded as a true hydrate of the alpha form. It is referred to as alpha hydrate.

The anhydrous form of alpha lactose may be prepared by desiccation of the hydrate. It has never been prepared by direct crystallization. The

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beta form of lactose is the stable modification crystallizing from aqueous solutions at temperatures above 93° C. It forms anhydrous crystals; no hydrate is known.

INTRODUCTION

The control of the crystallization of lactose is of considerable industrial importance. It is necessary to prevent crystallization in ice cream, because it produces the defect known as sandiness (1, 35). It is necessary to prevent crystallization in spray milk powder in order to avoid caking (27). Moreover, the solubility of the powder is greatly decreased when the lactose crystallizes. A large part of the lactose is crystallized in sweetened condensed milk, and conditions are so regulated as to secure the smallest crystals possible. In the manufacture of lactose it is desirable to secure a maximum yield of crystals in a minimum of time, and to secure crystals which may be readily washed with a minimum of loss. The manufacture of each of these products presents crystallization problems of its own, and some of these problems have not yet been satisfactorily solved.

Because lactose is a mutarotating sugar, its solubility varies with time. This was recognized by Urech (28) in 1883, and was studied in detail by Hudson (8, 9). Moreover, lactose forms supersaturated solutions quite readily (3). These facts make a study of the crystallization of lactose more difficult, but more interesting. The results of such a study are reported in this section.

A study of supersaturated solutions must be based upon a knowledge of the true solubility of lactose. Gillis (7) has published a critical review of the values reported in the literature, together with some original data of his own. Sharp (22, page 149) has published a table of interpolated values from -30 to 100° C. at 10° intervals. His figures are based upon the data of Hudson and of Gillis.

The phenomena of crystallization have been studied by a number of workers. Unfortunately for our purpose, much of the work has been done on crystallization of melts of pure compounds. For the most part, the studies have dealt with one component systems but a lactose solution contains at least two components, if not three, and it is not known, for example, how many components should be considered in the crystallization of lactose in ice cream. Nevertheless much can be learned by reviewing the results obtained by those who worked with the more simple systems.

Supersaturated solutions have been an object of interest to many. Ostwald (21) reported that supersaturated solutions were of two kinds: those which required seeding before crystallization could take place, and those which could crystallize spontaneously. He termed the first class metastable, and the second class, representing a higher degree of supersaturation, he termed labile. Ostwald's nomenclature has been accepted by many but

there has been much dispute as to whether there is any real difference between the metastable and the labile states.

Jones and Shah (13) as a result of their study of solutions of KCl, KBr and KI believed in the existence of a definite metastable zone. Leighton and Peter (14), who worked with lactose, reported a "supersolubility curve" located 30° C. below the true solubility curve. However, Young and his coworkers (32, 33, 34) have presented considerable evidence to show that the boundary of the labile zone is not a definite one, but that its apparent position is dependent upon the degree of mechanical shock to which the solution is subjected. Jaffe (11) also reported the boundary of the labile zone an indefinite one. By repeated filtration and recrystallization, he was able to widen the metastable region. He attributed this to the removal of insoluble particles which acted as crystal nuclei.

The existence of metastable and labile zones may be explained in terms of the results of Tammann (26). He has shown that crystallization is dependent upon two independent factors; first, the appearance of crystal nuclei; second, the rate of growth of nuclei after they appear. He has also shown that as a melt is cooled below its freezing point, the probability of nuclei formation rises gradually to a maximum and then returns to zero. This last fact explains the apparent stability of glasses. They are so greatly supercooled that the probability of nuclei appearing is very small. According to Tammann, the velocity of crystal growth also passes through a maximum and approaches zero with sufficient supercooling. Now, if we say that the metastable zone is the region where spontaneous crystallization will not occur, we must consider it as the zone where the probability of nuclei formation is practically zero. On further cooling, the probability of nuclei formation increases and the solution passes into the labile zone where crystallization may be expected in a reasonable length of time. On still further supercooling, the chances of nuclei appearing decrease again, and we have solutions which may be considered as being in a glassy state. According to this view, there can be no sharp distinction between the labile and the metastable solutions. However, if the temperature coefficient of probability is sufficiently great, then there might appear to be a sharp line dividing the two zones.

It must be remembered that the degree of supersaturation is not the only factor governing the appearance of nuclei. The effect of mechanical shocks is often very great. Moreover, Richards (23) has demonstrated that something having the properties of a crystal nucleus may exist in solution for considerable periods of time. No evidence is available to show that this is true of lactose solutions, but it is worth investigating. It is interesting to note that Bothell (1) raised this question as far back as 1920 when he remarked that if one accepted the opinions which were current regarding the crystallization of lactose in ice cream, one would have to believe that

lactose solutions which had been crystallized once, were more readily crystallized a second time. There are a number of observations recorded in the literature which apparently support a theory of crystal nuclei resulting from the incomplete solution of crystals. As examples of this, Jones and Shah (13) found it necessary to boil solutions of alkali halides for ten minutes in order to destroy all crystal nuclei, and Miers and Isaac (20) found it necessary to heat solutions of sodium chlorate in boiling water for 48 hours in order to prevent premature crystallization. If it could be shown that lactose solutions must be heated, even after all lactose is in solution, to destroy these "ghosts" of crystals which induce crystallization on cooling, many anomalous results of crystallization might be explained.

The rate of growth of crystals is independent of the formation of nuclei. It is governed by the rate of diffusion to the crystal, and by the speed with which molecules can be oriented into the crystal lattice. (Marc (16, 17, 18, 19) has shown that the process of orientation can be distinguished experimentally from that of diffusion.) In the case of the crystallization of lactose, the rate of transformation of beta to alpha lactose may be a factor during the later stages (8). Sharp (22, page 153) has calculated the maximum velocity of crystallization at various temperatures as limited by this last factor. The influence of pH upon crystallization velocity has been measured experimentally by Jenkins (12) and by Troy and Sharp (27). Whittier and Gould (31) have published some experimental results showing the rate of crystallization when the solutions were agitated so vigorously that diffusion was a minor factor. But there is still need for information regarding the crystallization of lactose in the absence of agitation.

Marc (18, 19) showed that the presence of a third substance may influence the rate of crystallization from solution. Leighton and Peter (14) studied the influence of dyes upon the crystallization of lactose. Only three out of thirty-nine seemed to have any effect at all, and their action was slight. Fujimoto (5) reported that none of the certified dyes had any appreciable effect upon the crystallization of lactose, though sucrose and gelatine seemed to inhibit crystallization. On the other hand, Dahle (2) found no evidence that other sugars, or gelatine, had any influence on the crystallization of lactose in ice cream. He believed that the governing factor was the lactose content of the mix. This view seems widespread in spite of the fact that the concentration of lactose in frozen ice cream is independent of its concentration in the mix.

This study of supersaturated solutions was pursued with several objects in mind. It was desired to repeat the work of Leighton and Peter on supersolubility, without agitation, to determine whether there is a sharp line between the metastable and labile zones, and if it were found to locate it as definitely as possible. It was desired to determine at what degree of supercooling the probability of nuclei formation passed through a maxi-

mum, and to extend the investigation into the zone of glasses, if that were possible. It was hoped, also, that information might be gained regarding the rate of growth of individual crystals at various degrees of supercooling.

EXPERIMENTS

Leighton and Peter determined the degree of supercooling necessary before one crystal could initiate a general formation of crystals when the solution was shaken. In this study, efforts were made to determine the degree of supercooling necessary for nuclei to appear spontaneously, and to estimate the number which appeared. This made it necessary to prevent a general seeding of the solution by the first crystal to appear. Two methods were used to accomplish this: first, crystallization of lactose solutions in slender glass tubes; and second, crystallization in the presence of a gel which held the crystals in the place where they first appeared.

In the first experiments, lactose and water were weighed into a series of glass tubes, approximately two millimeters inside diameter, which were then sealed carefully. After heating in boiling water until all of the lactose was dissolved, the tubes were stored horizontally in a room kept at a constant temperature. When crystals appeared in these tubes, they fell to the side, but their path was so short that little opportunity was given for them to initiate a general crystallization. These tubes were examined at intervals with a hand lens, and when possible, the number of crystals or of clusters was determined.

This method had the disadvantage that only very small volumes of solutions could be used, approximately 0.3 grams in each tube. For this reason, it was feared that crystallization might not occur in some of the experiments where it would take place in a larger volume of solution under the same conditions. However, several experiments were carried out by this method. The results of one experiment, where the crystallization was allowed to take place at 30° C., are shown in table 1. The results of these experiments are in agreement with those of Leighton and Peter who found that solutions must be supercooled 30° C. in order to initiate crystallization. However, it might be stated that in another experiment, tubes 15, 12 and 11 did not crystallize after 68 days at 0° C. where the supercooling was as great as 53° C. Tube 8 contained only one cluster at that time. These results indicate that the supersolubility curve is at a higher degree of supercooling in the case of the more dilute solutions.

In order to work with larger volumes of solution, another method was devised. Preliminary experiments indicate that 0.7 per cent of agar agar had no appreciable effect upon the crystallization of lactose, and it gave a gel firm enough to hold crystals in suspension. (Friedman (4) found that the diffusion of lactose was retarded only slightly in such a gel.) Accordingly, a 0.7 per cent solution of agar agar was prepared and carefully

filtered. The lactose was dissolved in this solution, and from the weights of the two components, the temperature of saturation was estimated using the table of solubilities compiled by Sharp (22, page 149). Ten cc. portions of these solutions were placed in test tubes and covered with vaseline to prevent evaporation. The test-tubes were heated for three minutes in boiling water to destroy all crystal nuclei, and then placed in thermostats maintained at suitable temperatures. In the case of the tubes held at 40° C. and at 50° C., it was necessary to cool the tubes momentarily to 37° C., to allow the agar agar to set. In order to reduce experimental errors, five tubes were used for each solution at each temperature.

Figure 1 shows the solubility curve of lactose, and the mean time ex-

TABLE 1

The appearance of crystal nuclei at 30° C., in solutions saturated at various temperatures

TUBE NUMBER	SATURATION TEMPERATURE OF	NUMBER OF CRYSTALS PRESENT				
		10 hours	34 hours	58 hours	136 hours	360 hours
2	96° C.	TMC	TMC	TMC	TMC	TMC
7	90	TMC	TMC	TMC	TMC	TMC
10	86	TMC	TMC	TMC	TMC	TMC
3	84	29-20	TMC	TMC	TMC	TMC
4	80	38- 3	TMC	TMC	TMC	TMC
1	75	22- 1	TMC	TMC	TMC	TMC
14	66	1	2	30- 2	TMC	TMC
13	61	1	1	0	0	TMC
9	54	0	0	0	0	0
15	53	0	0	0	1	0
12	51	0	0	0	0	0
8	46	0	1	1	0	0
11	46	0	0	1	0	0

TMC = too many to count.

Double numbers = single crystals first, clusters second.

pressed in hours for crystallization to commence at various degrees of supercooling. In one or two cases, one tube out of a set failed to crystallize even after the lapse of three thousand hours. Such tubes were disregarded in calculating the mean time for nuclei to appear. In the great majority of cases, the agreement of duplicates was quite satisfactory. The cases where no crystals appeared in any one of the set of five tubes after 3000 hours are designated by an interrogation mark. The tubes were examined for the presence of crystals by revolving before a bright light. The reflection of light from the crystals faces made possible the detection of even minute crystals.

A study of these data indicates that the higher the initial concentration of the solution, the less supercooling is possible before nuclei formation

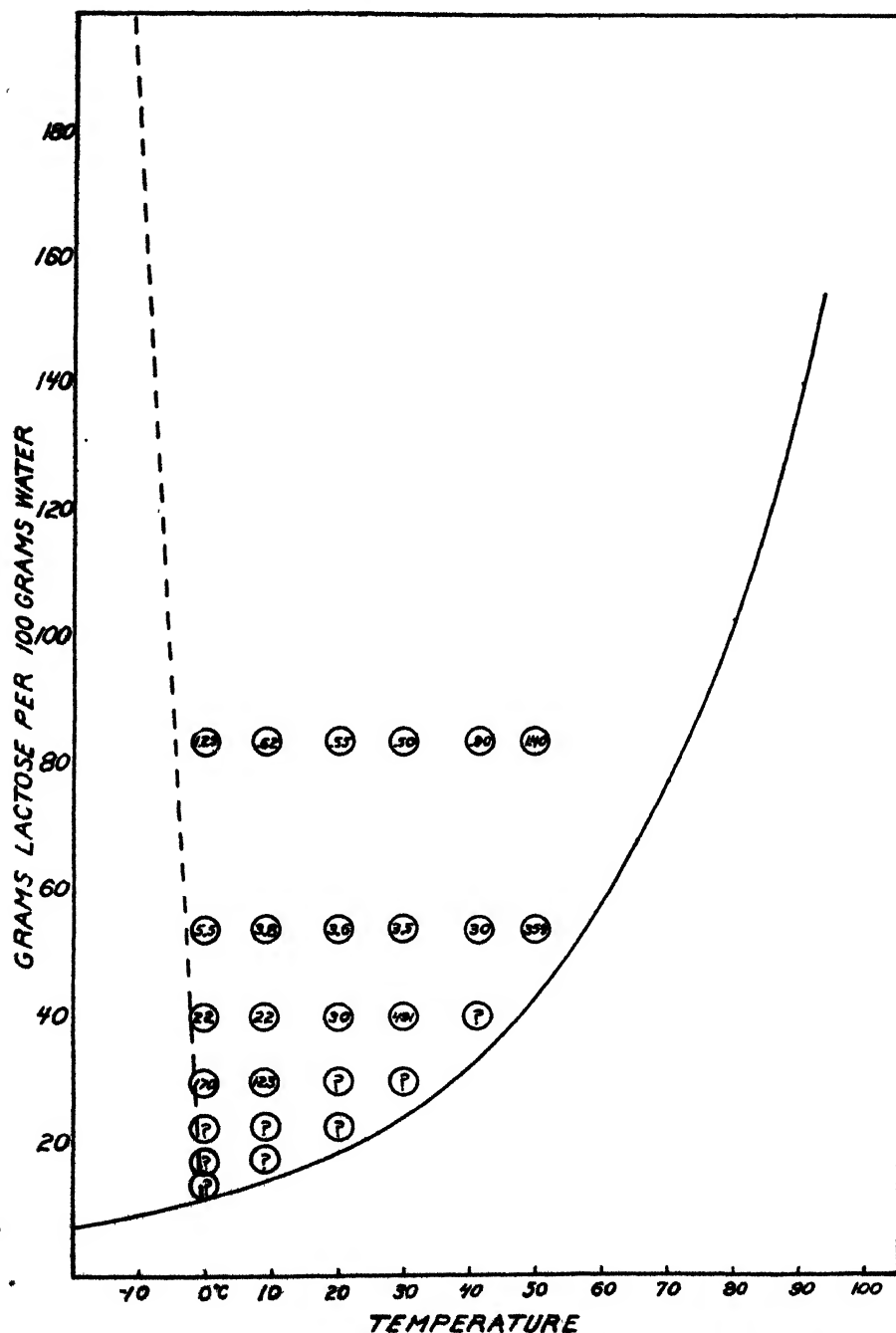


FIG. 1. THE SOLUBILITY CURVE OF LACTOSE, AND THE HOURS REQUIRED FOR CRYSTALS TO APPEAR AT VARIOUS DEGREES OF SUPERCOOLING.

† indicates no crystals after 3000 hours.

begins. This is in agreement with the results obtained by the other procedure. The data also show that the probability of nuclei formation is very small with slight degrees of supercooling, but it rises to a maximum and then falls again as the supercooling is increased. This fall is shown quite plainly in the case of the solutions saturated at 77° C. and at 57° C. It was not possible to cool the other solutions sufficiently to demonstrate this effect. These data also substantiate the belief that there is no real difference between the labile and the metastable zone with respect to spontaneous crystallization. There is no region of supersaturation which can be considered as permanently stable, although the probability of crystallization taking place may be very small.

Table 1 shows that the tendency to form clusters increases with the degree of supercooling. Examination of the test tubes indicated that, in general, there is a greater tendency to form clusters at low temperatures, with a given degree of supercooling, and that clustering increases with the degree of supercooling. This may explain the results of Leighton and Peter (14). They may have measured the cluster forming tendency instead of the tendency toward spontaneous nuclei formation. When the supercooling exceeded a certain value, new crystals would begin to form on the surface of the parent crystal and these would be broken away by the agitation to form new nuclei for crystal growth. If this is the true explanation of their results, then their data are applicable to those cases where there is vigorous agitation during crystallization but not elsewhere.

It is of some interest to extend the results shown in figure 1, to the case of lactose solutions held at temperatures below zero. Even though pure aqueous solutions of lactose can scarcely be expected to behave in the same manner as ice cream, yet some valuable suggestions may be obtained. We will assume that the water crystallizes out promptly on cooling. The first point to be considered is that after ice begins to form, the concentration of the solution is independent of its original concentration and depends only upon the temperature. Furthermore, after the ice begins to form, the unfrozen syrup may be considered as though it were a solution saturated at a much higher temperature than was originally the case. It is possible to estimate the approximate composition of the unfrozen lactose syrups at various temperatures by means of the known molecular depression of the freezing-point. The dotted line of figure 1, shows the approximate concentration of such syrups at various temperatures. It is apparent, at once, that the experimental data fall in a quite different part of the diagram from that which represents the conditions in an ice cream hardening room. However, we may say that at temperatures only a few degrees below freezing, the chances of spontaneous crystallization do not seem very great. With further supercooling, the probability of crystallization increases and must pass through a maximum although it is not possible to determine the posi-

tion of that maximum from the data now available. At still lower temperatures, the solution would become more stable since it is moving to the left on the chart while the point of maximum nuclei formation seems to be moving toward the right as the concentration increases.

One other point of interest might be considered in connection with figure 1. Let us suppose that a solution in equilibrium at -30°C . is warmed to -20°C . This might be done in either of two ways. If the temperature change occurs very slowly, ice would melt as the temperature rises, diluting the solution. The point on the diagram representing the solution would move downward along the dotted line to the new temperature. On the other hand, if the solution were warmed more rapidly than the melting ice could diffuse into the syrup, then the point representing the system would not move down along the line, but would move directly out to the -20°C . abscissa. It is not yet possible to predict the relative probabilities of nuclei formation at these two points, but if it should happen to be much greater in the second case than in the first, then the use of the term "heat shock" would be justified, and the need for preventing rapid fluctuations in storage temperature would be explained.

In the case of ice cream, the effect of the sucrose must be considered. In frozen ice cream, the total concentration of sugars is independent of the original sugar concentration and depends only upon the temperature. The concentration of lactose, however, would depend upon the ratio of lactose to sucrose as well as upon the temperature. In an average ice cream mix, the concentration of lactose in water would be only about one-fourth as great as the concentration in a pure lactose solution at the same temperature.

In a frozen mix, the concentration of lactose depends upon the lactose sucrose ratio. This fact might well be emphasized for it is one of the most important factors governing sandiness in ice cream. Furthermore this effect of sucrose has been overlooked entirely by those who have investigated the effect of sucrose upon the crystallization of lactose above the freezing point.

These experiments on crystallization also gave information regarding the rate of crystal growth at various degrees of supercooling in the absence of agitation. Accurate measurements were not possible, without disturbing the original experiment; but observations showed that if the solutions were cooled too much, the rate of crystal growth was greatly diminished, figure 2. The maximum rate of crystallization was at approximately 30°C . This agrees, in general, with the calculations of Sharp (22, page 153), and with the experiments of Whittier and Gould (31). In their cases, the limiting factor was the rate of transfer of beta to alpha lactose. However, in these experiments, the crystallization was so slow that mutarotation velocities must have been of secondary importance. The slow growth at the lower

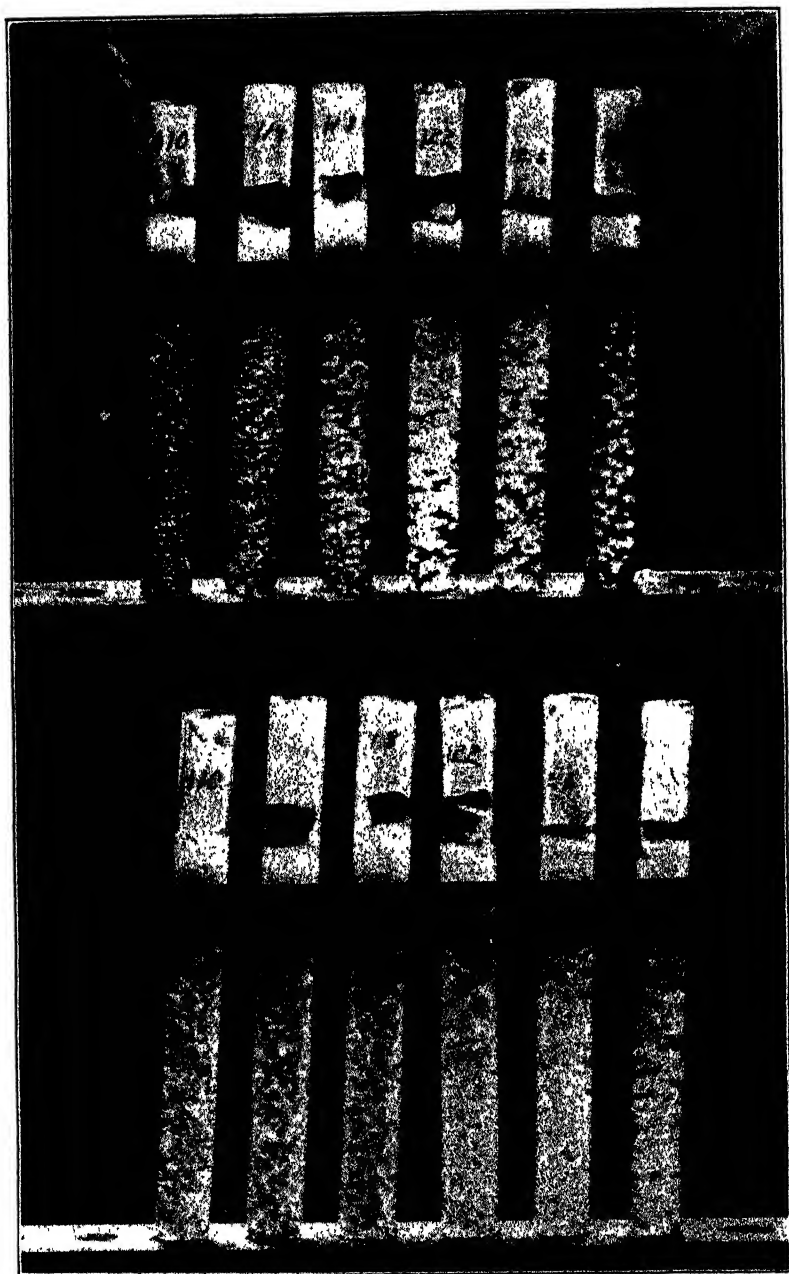


FIG. 2. THE EFFECT OF TEMPERATURE UPON THE RATE OF CRYSTAL GROWTH.

Two photographs of six tubes containing a lactose solution saturated at 77° C., each tube having been stored at a different temperature. From left to right, tubes were stored at 0°, 10°, 20°, 30°, 40°, and 50° C. The photographs were taken after 18.5 and 70 hours.

temperatures was probably due to a lower diffusion velocity of lactose in solution, and to a lower speed of orientation of molecules into the crystal lattice. Both of these factors would increase enormously in importance when a part of the water was frozen and therefore it seems reasonable to assume that in ice cream, the slow speed of mutarotation is of little importance. Mutarotation does place an upper limit to the rate of crystallization of lactose, but that maximum rate is probably never approached in ice cream. In any case, even without mutarotation, it would be possible to have two or three per cent of sand in ice cream which would be sufficient to render it unmarketable.

In spray powders, the lactose is not usually crystallized, yet the water content is so low that the solution is practically a solid (27). The same must be true of ice cream at storage temperatures. It is not possible to prepare such concentrated solutions by dissolving lactose directly in water, therefore other methods were devised by which they could be prepared in the laboratory for study.

According to theory, these glasses might be prepared by cooling a concentrated lactose solution rapidly enough to pass through the metastable and labile zones before crystallization began. The glasses could then be concentrated further, if desired, by desiccation at low temperatures. In practice, about eight grams of lactose was placed in a 250 cc. Erlenmeyer flask with about 15 cc. of water. The solution was then boiled down over a free flame until there was imminent danger of crystallization. The flask was then connected to a vacuum oven containing a tray of calcium chloride. When the stop-cock was opened, the lactose solution was converted into a foam which became solid in a few seconds because of the rapid cooling and evaporation. The vacuum pump (Hyvac) was allowed to run about one-half hour before the flask was disconnected. Then a glass marble was placed in the flask and it was tightly stoppered. The flasks used were of very heavy pyrex glass. With careful shaking, it was possible to reduce the glassy lactose to a fine powder which could easily be transferred to another container for storage.

It might be mentioned that success in the preparation of lactose glasses by this method seemed to depend partly upon the flask used. In some flasks, crystallization invariably began too soon, while boiling off the excess water. There was no visible difference in the nature of the glass surfaces which could account for this.

Under the microscope, these lactose preparations resembled ground glass. When examined by polarized light, some of the preparations seemed entirely free from crystalline material. Other batches showed a small amount of doubly refracting material. These glasses absorb moisture rapidly from the air, and under the microscope, they appear to melt down to a liquid and then recrystallize into solid cakes—see figure 3. Schmoeger (24) observed

this behavior of lactose more than 50 years ago. More recently, Troy and Sharp (27) have suggested that this is the explanation of the caking of milk powders and of the peculiar hydration-dehydration curves found by Supplee (25) in his study of milk powders.

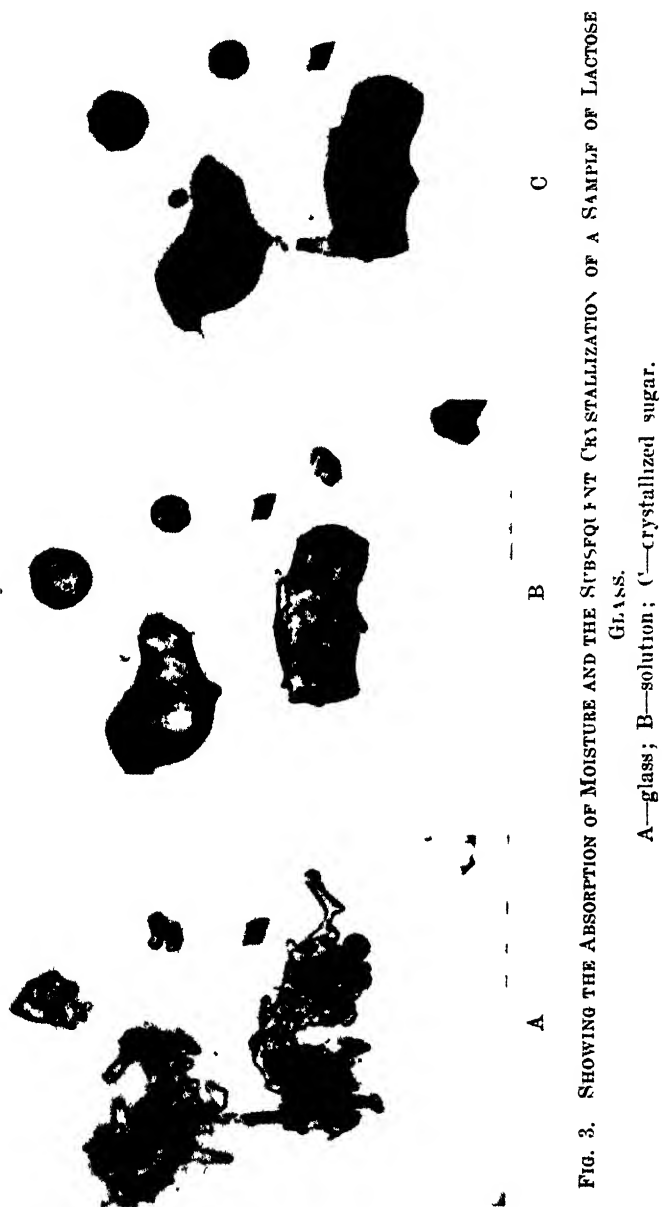
For some purposes, glasses were also prepared by drying lactose solutions rapidly on a Mojonnier machine in accordance with the procedure used for solids in milk. This method had the disadvantage that only small amounts, 0.2 gram, could be prepared at one time without having crystallization occur, and it was practically impossible to recover the glass from the dish.

The moisture content of the glasses, as prepared by the first method, ranged from about 3 per cent to about 8 per cent. This was determined in two ways. First, by weighing the flask plus lactose, and later weighing the flask plus glass; and second, by drying the glass in a vacuum over P_2O_5 . These moisture contents correspond to lactose concentrations as high as 3200 grams of anhydrous sugar per 100 cc. of water. High as this figure seems to be, it corresponds very well to conditions as they exist in a spray powder.

Lactose glasses give up most of their water more rapidly than does alpha hydrate. This was clearly shown by one experiment in which samples of powdered lactose glass, and of alpha hydrate, having approximately the same particle size were dried in a vacuum at room temperature over P_2O_5 . Figure 4, shows the rates of loss in weight.

The increased rate of loss by the glass probably accounts for the speed with which a Mojonnier solid's residue can be dried to an apparently constant weight. However, since lactose glass is a solution, constancy of weight does not mean complete dryness but merely the establishment of an equilibrium. The lactose glass used in this experiment appeared to contain only 6.0 per cent of moisture but it continued to lose weight, very slowly, until a total loss of 6.65 per cent was reached. At this point, the vacuum oven was heated to 100° C. and held at that temperature for two weeks. After this treatment, the total loss was 7.09 per cent. The glass showed no discoloration in spite of the treatment which it had received. On the other hand, the crystalline lactose had lost only 5.09 per cent of its original weight but it had become brown.

Lactose glasses of low moisture content may be kept in a desiccator for indefinite periods without crystallization. If exposed to the air, they take up water until crystallization begins, whereupon they lose their excess moisture. Usually alpha hydrate is formed, but in some cases, the product was apparently beta lactose. Figure 5 shows some typical hydration-dehydration curves of pure lactose glasses. The glasses were prepared by dissolving a known weight of lactose in two cc. of water and then drying the solution rapidly by the Mojonnier procedure (evaporation of free water



on a hot plate at 185°C ., followed by drying in a vacuum oven at 100°C .). The dish containing the residue was weighed on a chainomatic balance and then a beaker containing water was placed in the balance case. The door of the balance was left raised about one centimeter. Weighings were made every ten minutes until the changes in weight became so small that the periods were extended. In some cases, the final weights approached 100 per cent of the original weight, indicating the formation of alpha hydrate. In other cases, the final weights were much lower indicating the formation of beta lactose. In different experiments percentages of 96.02, 96.56 and 95.15 were obtained. In each of these cases, the sample was still losing weight when the experiment was discontinued. It seemed probable that they were approaching the limiting value of 95 per cent corresponding to the formation of beta lactose. Direct evidence for the presence of beta lactose is lacking, but there is no reason why beta lactose might not be formed under these conditions, particularly if the glass became seeded with beta during the initial heating period.

It was hoped that the hydration curves would indicate how much moisture was necessary before crystallization could take place at room temperature. However, no agreement was found between the maximum moisture contents reached in the different experiments. This was not surprising, since the method used measured the mean moisture content of the sample,

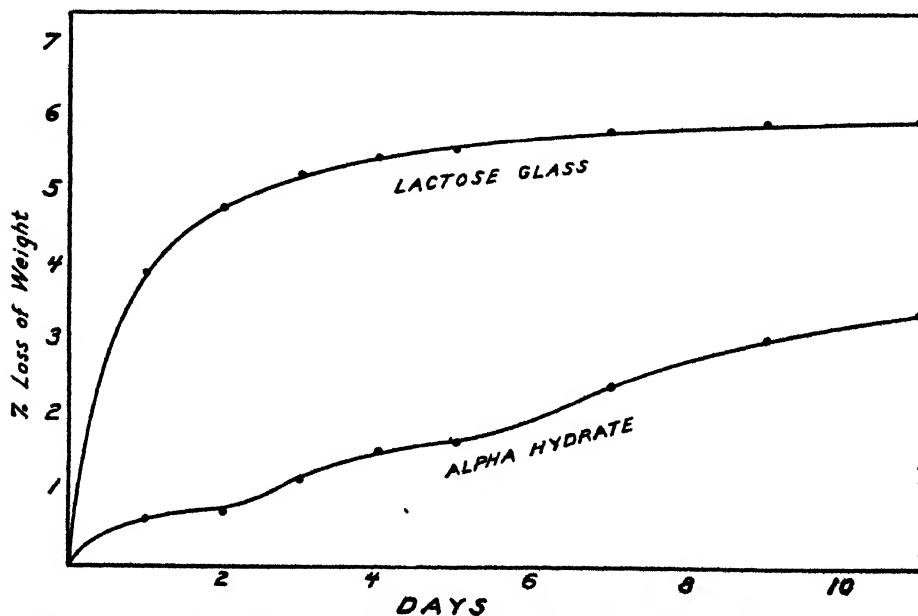


FIG. 4. COMPARATIVE RATES OF DEHYDRATION OVER P_2O_5 AT ROOM TEMPERATURE, UNDER VACUUM.

Actual moisture contents: glass, 7.09%; hydrate, 5.09%.

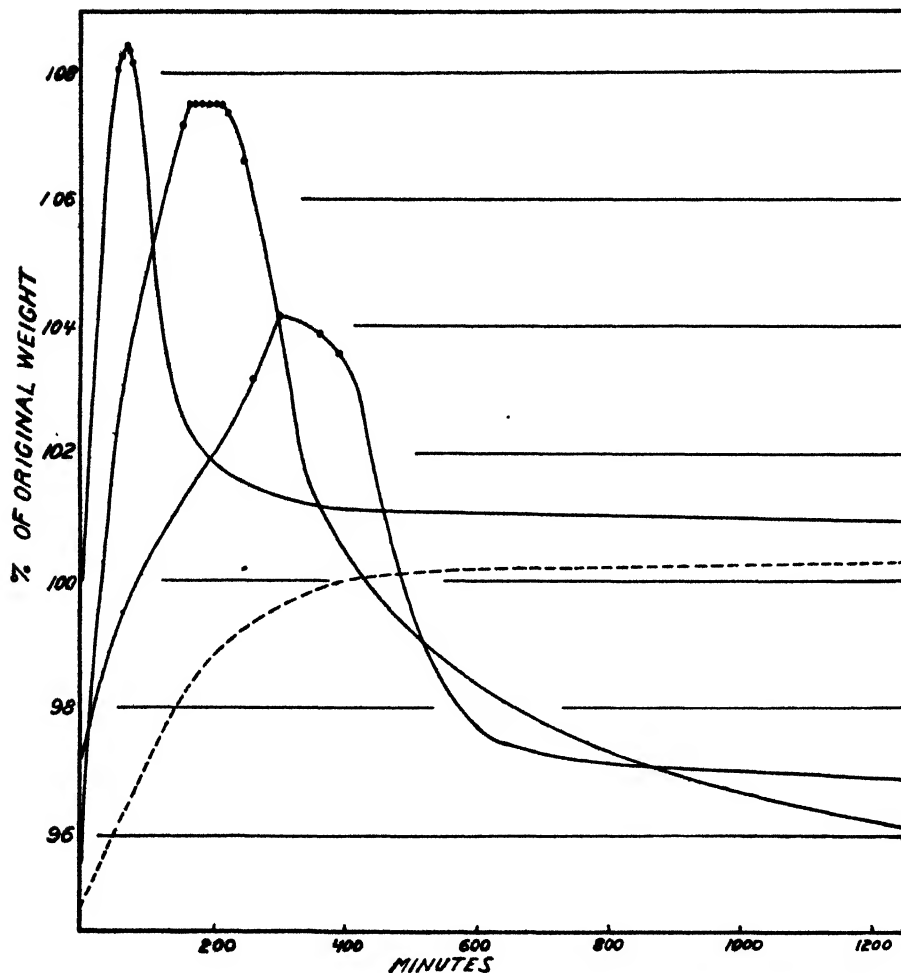


FIG. 5. THE HYDRATION AND DEHYDRATION OF LACTOSE AT CONSTANT HUMIDITY.
Solid lines represent glasses; dotted lines, alpha anhydride.

while crystallization was determined by the moisture content of the most dilute part of the glass. For that reason, high values of moisture content at the beginning of crystallization are more significant than low values. It might be possible to obtain concordant results by allowing the glass to take up moisture very slowly, but it was not considered that such a method would prove very satisfactory and it was not tried.

The crystallization of lactose may be brought about by the addition of alcohol to aqueous solutions of the sugar. This method was frequently employed, in the preparation of pure lactose, before the complex nature of lactose solutions was understood. As an example of this, Walker (29), in his study of the determination of reducing sugars, prepared a "precipi-

tated" lactose $C_{12}H_{22}O_{11} \cdot \frac{1}{2} H_2O$. Accordingly, he published tables for the reducing power of hydrated, anhydrous and of precipitated lactose. His product contained 2.43 per cent of water.

Casual observation indicated that "precipitated" lactose must vary a great deal in its composition, which is a fact of some importance in interpreting the results of some of the early work on lactose. In order to confirm this, a microscopic examination was made of the crystals obtained by adding different volumes of 95 per cent ethanol to saturated solutions of lactose. When the proportion of alcohol was low, only alpha hydrate was precipitated. When larger proportions of alcohol were used, beta lactose was also thrown out of solution. Unless a very large volume of alcohol was used, it seems certain that the "precipitated" lactose would always contain an excess of the alpha modification. This seems to be true of Walker's preparation if we may judge by the moisture content.

The fact that precipitated lactose may vary greatly in composition was shown clearly by an experiment in which 5 volumes of 95 per cent ethanol was added to a saturated solution of lactose at approximately $0^\circ C$. The resulting solution was analyzed for total solids at 15 minute intervals. The data are given in table 2. The concentration fell rapidly at first, due to the precipitation of alpha lactose. Then, after an interval of thirty minutes without change, beta lactose began to precipitate though the separation of beta was not as rapid as that of alpha.

TABLE 2

The precipitation of lactose after the addition of five volumes of 95 per cent ethanol to one volume of saturated aqueous solution at a temperature of $0^\circ C$.

TIME IN MINUTES	PER CENT OF SOLIDS IN SOLUTION
0	2.08
15	1.13
30	1.10
45	1.10
60	0.877
75	0.526
90	0.388
105	0.289
120	0.249
180	0.210

SUMMARY

Lactose solutions may be supercooled greatly without crystallization taking place. The degree of supercooling necessary for crystallization to occur, in the absence of agitation, is less for concentrated solutions than for dilute solutions.

There is no sharp line dividing the metastable and labile zones in the case of supersaturated lactose solutions.

As a solution of lactose is supercooled, the rate of nuclei formation passes through a maximum. The temperature of most rapid nuclei formation is higher in the case of concentrated solutions.

The rate of crystal growth passes through a maximum as the temperature is lowered. At low temperatures, the rate of crystallization, in the absence of agitation, is so slow that mutarotation cannot be the limiting factor.

Lactose solutions may be prepared which are so greatly supersaturated as to resemble solids. Such glasses are stable at room temperature, if protected from moisture.

Lactose glasses are hygroscopic, absorbing moisture from the air until sufficiently dilute for crystallization to take place.

Lactose glasses are supersaturated with respect to both alpha hydrate and beta anhydride. Either modification may appear when such glasses crystallize.

When desiccated, lactose glasses lose a part of their moisture quickly, coming to an apparently constant weight. This probably accounts for the success of the Mojonnier method of determining total solids in milk. The removal of the last portions of water from such glasses is very slow.

Lactose precipitated by alcohol is not equilibrium lactose, though it may resemble it closely. Alpha lactose is more readily precipitated from solution by alcohol than is beta lactose. Consequently, precipitated lactose varies in composition. It usually contains a greater proportion of alpha lactose than is present in the equilibrium mixture.

REFERENCES

- (1) BOTHELL, F. H. Facts about sandy ice cream. *Ice Cream Trade Journal* 16 (2): 55-56. 1920.
- (2) DAHLE, CHESTER D. Sandy ice cream. *Proceedings World's Dairy Congress* 1: 500-10. 1924.
- (3) DUBRUNFAUT, ———. Note sur le sucre de lait. *Compt. rend.* 42: 228-33. 1856.
- (4) FRIEDMAN, LEO. Structure of agar gels from studies of diffusion. *J. Am. Chem. Soc.* 52: 1311-4. 1930.
- (5) FUJIMOTO, A. T. Some factors influencing the rate of crystallization of lactose in ice cream. M.S. Thesis. Ohio State University. 1926.
- (6) GILLIS, J. Nouvelles recherches sur le sucre de lait. *Rec. trav. chim.* 39: 88-125. 1920.
- (7) GILLIS, J. Solubilité du sucre de lait. *Rec. trav. chim.* 39: 677-8. 1920.
- (8) HUDSON, C. S. The hydration of milk sugar in solution. *J. Am. Chem. Soc.* 26: 1065-82. 1904.
- (9) HUDSON, C. S. Further studies on milk sugar. *J. Am. Chem. Soc.* 30: 1767-83. 1908.
- (10) HUDSON, C. S., AND HOCKETT, R. C. A novel modification of lactose. *J. Am. Chem. Soc.* 53: 4455-56. 1931.
- (11) JAFFÉ, GEORGE. Studien an übersättigten Lösungen. *Z. physik. chem.* 43: 565-94. 1903.

- (12) JENKINS, J. D. The effect of various factors upon the velocity of crystallization of substances from solution. *J. Am. Chem. Soc.* 47: 903-22. 1925.
- (13) JONES, B. M., AND SHAH, P. G. The spontaneous crystallization of solutions of potassium chloride, potassium bromide, and potassium iodide. *J. Chem. Soc.* 103: 1043-52. 1913.
- (14) LEIGHTON, A., AND PETER, P. N. Factors influencing the crystallization of lactose. *Proceedings World's Dairy Congress* 1: 477-85. 1924.
- (15) LOWRY, T. M. Studies of dynamic isomerism. III. Solubility as a means of determining the proportions of dynamic isomerides in solution. Equilibrium in solutions of glucose and galactose. *J. Chem. Soc.* 85: 1551-70. 1904.
- (16) MARC, ROBERT. Über die Krystallization aus wässerigen Lösungen. *Z. physik. Chem.* 61: 385-98. 1908.
- (17) MARC, ROBERT. Über die Krystallization aus wässerigen Lösungen, II. *Z. physik. Chem.* 67: 470-500. 1909.
- (18) MARC, ROBERT. Über die Krystallization aus wässerigen Lösungen, III. *Z. physik. Chem.* 68: 104-14. 1909.
- (19) MARC, ROBERT. Über die Krystallization aus wässerigen Lösungen, IV. *Z. physik. Chem.* 73: 685-723. 1910.
- (20) MIERS, H. A., AND ISAAC, FLORENCE. The refractive indices of crystallizing solutions with special reference to the passage from the metastable to the labile condition. *J. Chem. Soc.* 89: 413-54. 1906.
- (21) OSTWALD, W. Studien über die Bildung und Umwandlung fester Körper. I. Übersättigung und Überkaltung. *Z. physik. Chem.* 22: 289-330. 1897.
- (22) RAHN, OTTO, AND SHARP, PAUL FRANCIS. *Physik der Milchwirtschaft*. Paul Parey, Berlin. 1928.
- (23) RICHARD, WILLIAM T. Persistence and development of crystal nuclei above the melting point. *J. Am. Chem. Soc.* 54: 479-95. 1932.
- (24) SCHMÖGER, M. Ueber wasserfreien Milchezucker. *Ber.*, 14: 2121-6. 1881.
- (25) SUPPLEE, G. C. Humidity equilibria of milk powders. *JOUR. DAIRY SCI.* 9: 50-61. 1926.
- (26) TAMMANN, G. Über die Abhängigkeit der Zahl der Kerne, welche sich in verschiedenen unterkühlten Flüssigkeiten bilden, von der Temperatur. *Z. physik. Chem.* 25: 441-79. 1898.
- (27) TROY, H. C., AND SHARP, PAUL FRANCIS. Alpha and beta lactose in some milk products. *JOUR. DAIRY SCI.* 13: 140-57. 1930.
- (28) URECH, F. Ursächlicher Zusammenhang zwischen Löslichkeits und optischer Drehungserscheinungen bei Milchezucker und Formulierung der Uebergangsgeschwindigkeit seiner Birotation in die Normale. *Ber.*, 16: 2270-71. 1883.
- (29) WALKER, PERCY H. The unification of reducing sugar methods. *J. Am. Chem. Soc.* 29: 541-554. 1907.
- (30) WHITTIER, E. O. Lactose, a review. *Chem. Reviews* 2: 85-125. 1925.
- (31) WHITTIER, E. O., AND GOULD, S. P. Speed of crystallization of lactose, galactose, glucose, and sucrose from pure solutions. *Ind. Eng. Chem.* 23: 670-3. 1931.
- (32) YOUNG, S. W. Mechanical stimulus to crystallization in supercooled liquids. I. *J. Am. Chem. Soc.* 33: 148-62. 1911.
- (33) YOUNG, S. W., AND CROSS, R. J. Mechanical stimulus to crystallization. II. *J. Am. Chem. Soc.* 33: 1375-88. 1911.
- (34) YOUNG, S. W., AND VAN SICKLEN, W. J. The mechanical stimulus to crystallization. *J. Am. Chem. Soc.* 35: 1067-78. 1913.
- (35) ZOLLER, H. F., AND WILLIAMS, O. E. Sandy crystals in ice cream: their separation and identification. *J. Ag. Research* 21: 791-5. 1921.

INFLUENCE OF HOMOGENIZATION ON THE CURD TENSION OF MILK*

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Since the work of Hill (1), 1923, considerable attention has been given to the study of soft curd milk. Although it is possible to modify the curd character of milk by heating, natural soft curd milk is at present practically the sole source of supply.

As early as 1916 Washburn and Jones (8) studied the values of different grades of milk for infant feeding using pigs as experimental animals. After studying the effect of homogenization they concluded, "The homogenization of the fat does not seem to be helpful, but the casein of the milk is sufficiently modified by the application of this process so that possible benefit may result from its employment." Ladd (4), 1915, Washburn (9), 1931, Wallace (7), 1932, and Weisberg *et al.* (10), 1933, report that homogenization lowers the curd tension of milk. In fact, the National Dairy Products Corporation, Inc., was granted a patent (6) in January, 1932, covering the production of soft curd milk by homogenization. Dr. Shrader (5), president of the Research Laboratories of the company, states that the patent office will probably cancel this patent as the company has declined to reply to a citation.

Just what relationship exists between the pressure of homogenization and the curd tension of milk has never been established. This work was undertaken in an effort to determine this relationship.

PROCEDURE

Milk used in this work was from cows in the University of Idaho dairy herd, composed of Holsteins and Jerseys. In no instance was any milk used from cows having any physical or bacteriological symptoms of mastitis.

Part I

Thirty gallons of milk were divided into six split batches. Each of the five-gallon batches was heated to 130° Fahrenheit and then homogenized at a selected pressure in either a single-stage or two-stage homogenizer.

Homogenization pressures of 500, 1,000, and 2,000 pounds were used. The two-stage homogenizer was operated with the second valve at three-fourths the pressure of the first valve.

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Curd tension was determined within one-half hour after homogenization by the method recommended by Hill (2). Ten trials were run.

Part II

Five gallons of milk were selected from each of three groups of three cows, one group producing relatively high, one medium, and one low curd tension milk. Milk with a curd tension above 60 grams was considered as relatively high curd tension milk; 30 to 60 grams, relatively medium curd tension; and below 30 grams, low or soft curd milk. Each lot of milk from the three groups of cows was heated to 130° Fahrenheit and homogenized at 1,000 pounds' pressure in a single-stage homogenizer.

Curd tension was determined as in Part I. Ten trials were run with each group.

PRESENTATION OF DATA

Part I

Effect of type of homogenizer and pressure of homogenization

The curd tension of milk was reduced by homogenization, using either a single-stage or a two-stage homogenizer at pressures of 500, 1,000, and 2,000 pounds (Table 1, Figure 1). Increases in homogenizing pressures progressively reduced the curd tension.

A pressure of 500 pounds on the single-stage and two-stage homogenizers reduced the curd tension 28.4 and 20.8 per cent respectively. The effect,

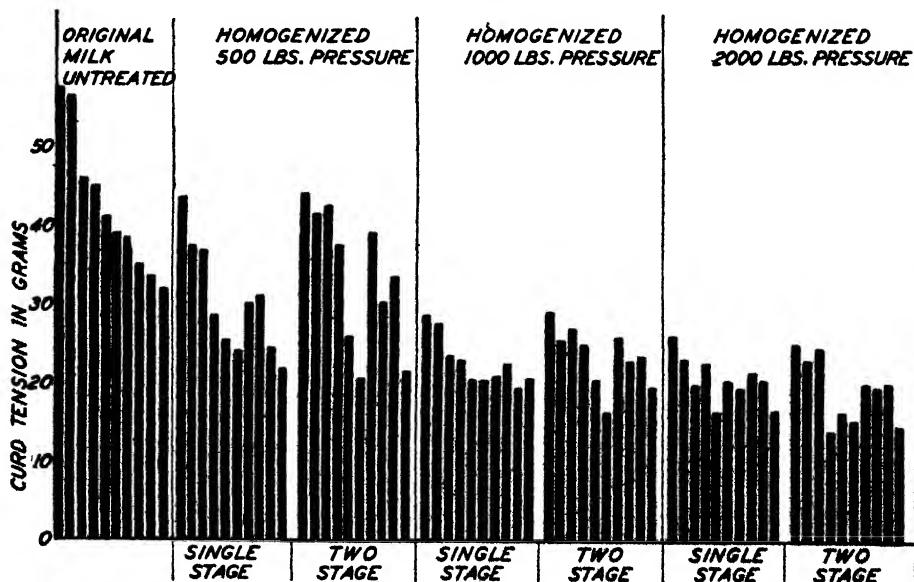


FIG. 1. EFFECT OF HOMOGENIZATION ON THE CURD TENSION OF MILK WHEN SINGLE-STAGE AND TWO-STAGE HOMOGENIZERS WERE USED AT DIFFERENT PRESSURES.

TABLE I
Effect on curd tension of single-stage and two stage homogenizers at different pressures

TRIAL NUMBER	ORIGINAL MILK	500 LBS. PRESSURE		1,000 LBS. PRESSURE		2,000 LBS. PRESSURE	
		Single Stage	Two Stage	Single Stage	Two Stage	Single Stage	Two Stage
Average (mean)	42.4 \pm 2.8	30.4 \pm 2.1	33.6 \pm 2.9	22.8 \pm 1.0	23.6 \pm 1.2	20.7 \pm 0.9	19.3 \pm 1.3
Standard Deviation	8.5 \pm 1.9	6.4 \pm 1.4	8.8 \pm 1.9	3.1 \pm 0.7	3.6 \pm 0.8	2.7 \pm 0.6	3.8 \pm 0.8
Per cent Decrease		28.4	20.8	46.4	44.3	51.3	54.6

TABLE II
Effect of homogenization on relatively high, medium, and low curd tension milk
(Single Stage Homogenizer, 1,000 lbs. Pressure)

TRIAL NUMBER	HIGH CURD TENSION GROUP		MEDIUM CURD TENSION GROUP		LOW CURD TENSION GROUP	
	Original	Homogenized	Original	Homogenized	Original	Homogenized
Average (mean)	67.9 \pm 2.7	35.7 \pm 2.6	46.2 \pm 1.9	28.5 \pm 1.4	38.6 \pm 1.8	27.4 \pm 1.4
Standard Deviation	8.3 \pm 1.9	7.9 \pm 1.8	5.6 \pm 1.2	4.3 \pm 0.9	5.4 \pm 1.2	4.3 \pm 0.9
Per cent Decrease	48.5		38.3		28.9	

however, was particularly noticeable when 1,000 pounds' pressure was used for the curd tension was decreased 46.4 per cent with the single-stage homogenizer and 44.3 per cent with the two-stage homogenizer. A further reduction in curd tension, approximately 5 and 10 per cent, or 2 and 4 grams respectively, resulted when the pressure on both homogenizers was increased from 1,000 to 2,000 pounds. The reduction in curd tension was not proportional to the increase in homogenization pressure.

There was apparently little difference between the single-stage and the two-stage homogenizers in their ability to reduce the curd tension. The single-stage homogenizers gave a slightly greater reduction in curd tension at both 500 and 1,000 pounds' pressure than did the two-stage homogenizer. At 2,000 pounds' pressure, however, the two-stage homogenizer caused a slightly greater reduction in curd tension.

These results demonstrate that: first, the curd tension of milk is reduced by homogenization at 500, 1,000, and 2,000 pounds' pressure; second, the percentage reduction in curd tension increases with an increase in homogenizing pressure, but the increase in percentage reduction of curd tension is not proportional to the increase in pressure of homogenization; and third, there is no appreciable difference in the efficiency of single and two-stage homogenizers in the reduction of the curd tension of milk at pressures used in this work.

Part II

Effect of homogenization on high, medium, and low curd tension milk

The results of homogenizing relatively high, medium, and low curd tension milk in a single-stage homogenizer at 1,000 pounds' pressure are shown in table 2, figure 2. A single-stage homogenizer was used in this phase of the work as it was the only one available at the time. The results, however, are comparable to those which may be expected from a two-stage homogenizer. A pressure of 1,000 pounds was used because it had been suggested by commercial workers and because there was little difference between the results obtained with 1,000 and 2,000 pounds' pressure.

Results indicate that the percentage reduction in curd tension increases with an increase in the curd tension of the unhomogenized milk. The average percentage reduction in 10 trials on relatively low, medium, and high curd tension milk was 28.9, 38.3, and 48.5 per cent respectively.

DISCUSSION OF RESULTS

Considerable interest has developed in the use of soft curd milk for infant feeding. If soft curd milk continues to merit the attention of milk distributors, more extensive developments along this line could be expected if curd tension could be regulated by processing the milk rather than selecting milk from cows naturally producing soft curd milk.

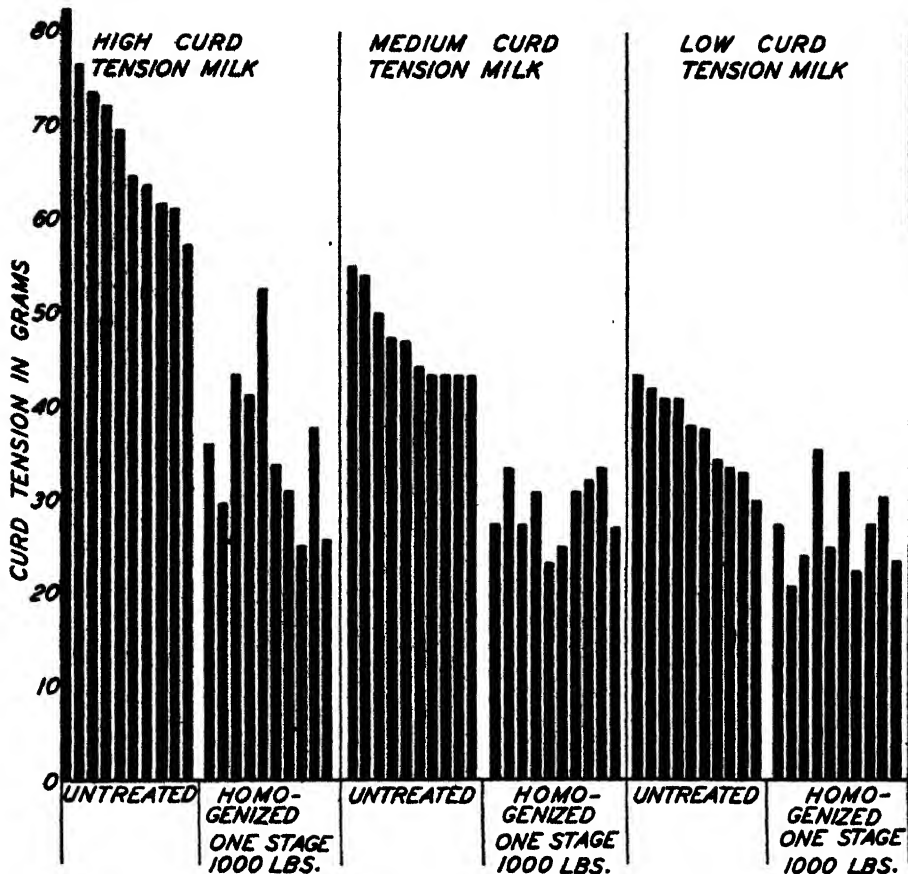


FIG. 2. COMPARISON OF HIGH, MEDIUM, AND LOW CURD TENSION MILK BEFORE AND AFTER HOMOGENIZATION AT 1000 POUNDS PRESSURE, WITH A SINGLE-STAGE HOMOGENIZER.

Data presented show that the curd tension of milk can be decreased by homogenization. There is apparently little difference between the single-stage and the two-stage homogenizers in their effectiveness in lowering the curd tension of milk. Increasing the pressure of homogenization decreases the curd tension, but the decrease in curd tension is not proportional to the increase in pressure of homogenization. If 500 pounds' pressure is used, a reduction of about 25 per cent in curd tension may be expected; with 1,000 pounds, a reduction of about 46 per cent; and with 2,000 pounds, about 53 per cent. Results obtained with pressures of 1,000 and 2,000 pounds agree with the work of Wallace (4).

Results also indicate that the higher the curd tension of the unhomogenized milk, the greater will be the percentage reduction in curd tension.

The reduction in curd tension is not great enough to make low curd tension milk out of milk with a curd tension above 60 grams, but will usually make low curd tension milk out of milk with a curd tension between 30 and 60 grams. Medium curd tension milk was reduced in curd tension sufficiently to fall in the low or soft curd class in 23 out of 31 instances.

Although Hill (3) questions the use of the homogenizer in the production of soft curd milk, this work indicates that homogenization has commercial possibilities and might be used for two purposes: first, to further lower the curd tension of low curd tension or soft curd milk; and second, to make soft curd milk out of milk with a curd tension between 30 and 60 grams.

SUMMARY

1. Homogenization at pressures of 500, 1,000, or 2,000 pounds reduced the curd tension of milk approximately 25, 46, and 53 per cent respectively.
2. Single-stage and two-stage homogenizers were equally effective in reducing the curd tension of milk.
3. The higher the curd tension of the original milk, the greater was the percentage reduction after homogenization.
4. Homogenization might be used to further lower the curd tension of soft curd milk or to make soft curd milk out of milk of medium curd tension (30 and 60 grams), or reduce high curd tension milk to medium curd tension.

REFERENCES

- (1) HILL, R. L. A test for determining the character of the curd from cow's milk, etc. *JOUR. DAIRY SCI.* 6: 509. 1923.
- (2) HILL, R. L. Soft-curd milk. Bulletin 227, Utah Agricultural Experiment Station. 1931.
- (3) HILL, R. L. A decade and a half of soft-curd milk studies. Circular 101, Utah Agricultural Experiment Station. 1933.
- (4) LADD, MAYNARD, M.D. Homogenized milk. *Boston Med. and Surg. Jour.* Vol. clxxiii, No. 1. Pp. 13-18. July, 1915.
- (5) SHRADER, J. H., President National Dairy Products Corporation, Inc., 1403 Eutaw Place, Baltimore, Md. Personal correspondence.
- (6) United States Patent Office—Patent Number 1,841,184. Process of Treating Milk. January 12, 1933.
- (7) WALLACE, C. Soft-curd milk. *Amer. Jour. Dis. Child.* 44: 1143-1144. 1932. Cited by Hill (3).
- (8) WASHBURN, R. M., AND JONES, C. H. Studies of the value of different grades of milk in infant feeding. Bul. 195, University of Vermont and State Agricultural College.
- (9) WASHBURN, R. M. Soft-curd milk. *The Milk Dealer* 21(3). December, 1931.
- (10) WEISBERG, S. M., JOHNSON, A. H., AND MCCOLLUM, E. V. Laboratory studies on the chemistry of soft curd milk. *JOUR. DAIRY SCI.* 16: 225-247. 1933.

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THE DIFFERENTIATION OF *STREPTOCOCCUS LACTIS* FROM *STREPTOCOCCUS FECALIS*

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Ayers and Johnson (1) have shown the great similarity between *Streptococcus lactis* and *Streptococcus fecalis* even when tested by the more modern methods used in the study of streptococci. So much alike are these organisms that it has been questioned whether or not they are in fact identical. Such a view is supported by the work of Demeter (2). Demeter states that the organism known as *Streptococcus lactis* may represent at most only a strain of the fecal streptococcus which frequently assumes a stabilized or habitat form in milk—a possibility suggested also by Ayres and Johnson. Beginning with Kruse (3), a number of older investigators, with less refined methods of differentiation, have believed that these organisms represent only one species.

In addition to general morphological, cultural and fermentative characteristics, the two organisms are similar in their action on blood, their ability to grow at low temperatures (below 10° C.), and in having strong reducing action, evidenced by a sharp reduction of litmus in milk prior to curdling. The one practical test which has served to differentiate the two species is growth at 45° C. At this temperature *Streptococcus lactis* does not grow while *Streptococcus fecalis* does. There is fairly abundant evidence that the maximum temperature for the growth of *Streptococcus lactis* is about 43° C. while the limiting temperature for *Streptococcus fecalis* is around 50° C. (Sherman and Albus, 5; Orla-Jensen, 4; Sherman and Stark, 6.)

It is desirable to know other differences which correlate with the maximum-growth temperatures in order to establish more definitely that these organisms represent two distinct species. For this purpose we have studied 27 cultures of *Streptococcus lactis*, isolated originally from milk and milk products, and 14 organisms of the "fecalis group." Of the latter 14 cultures, seven were typical *Streptococcus fecalis* while the other seven were the glycerol fermenting variety to which Orla-Jensen (4) has given the name of *Streptococcus glycerinaceus*. For the purposes of this paper, these two types will be referred to as *Streptococcus fecalis* or as the fecalis group, since they may be differentiated from *Streptococcus lactis* by the same tests.

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The following four tests were found to differentiate perfectly the cultures of *Streptococcus lactis* from those of the *Streptococcus fecalis* group:

(1) *Maximum growth temperatures.* As was to be expected from previous knowledge, none of the *lactis* cultures was able to grow at 45° C. and their maximum temperatures were found to range from 41 to 43° C. The *fecalis* cultures all grew vigorously at 45° C. and had maximum temperatures between 48 and 52° C.

(2) *Thermal death rates.* When placed in sterile skimmed milk and heated for 30 minutes at 65° C., all of the *lactis* cultures were killed while all of those of the *fecalis* group survived. As would of course be expected, when subjected to lower heat treatments the *fecalis* group survived in much greater numbers than did the cultures of *Streptococcus lactis*.

(3) *Limiting hydroxyl-ion concentration of growth.* When seeded in poured lactose nutrient agar plates (not streaked) adjusted to varying degrees of alkalinity it was found that *Streptococcus fecalis* is more tolerant to hydroxyl-ion concentration than is *Streptococcus lactis*. At a pH value of 9.6 all of the *lactis* cultures were entirely inhibited and all of those of the *fecalis* group grew rapidly. (The sterilized, melted and cooled agar was adjusted to the desired pH immediately before use.)

(4) *Inhibition of growth by sodium chloride.* In poured lactose nutrient agar plate cultures all of the *lactis* cultures were completely inhibited by 6 per cent NaCl and only three of the 27 cultures were able to grow in the presence of 5.5 per cent of this salt. On the other hand, all of the *fecalis* cultures grew vigorously in the same medium containing 6.5 per cent NaCl. (In these tests the NaCl was sterilized separately in concentrated solution and added to the melted agar medium before use.)

SUMMARY

Streptococcus lactis may be readily differentiated from *Streptococcus fecalis*.

Streptococcus lactis has a lower maximum growth temperature, a lower thermal death point, a lower alkaline limit for growth, and a lower tolerance for sodium chloride.

Although present conceptions of bacterial species may be profoundly modified as knowledge of dissociation and variation increases, based upon present criteria, there would appear to be ample basis for considering these organisms distinct species.

REFERENCES

- (1) AYERS, S. II., AND JOHNSON, W. T., JR. Jour. Infect. Dis., 34: 49. 1924.
- (2) DEMETER, K. J. Milchwirtschaftliche Forshungen, 8: 201. 1929.
- (3) KRUSE, W. Centralbl. Bakt., Abt. I, 34: 737. 1903.
- (4) ORLA-JENSEN, S. Mem. Acad. Roy. Sci. Lett. (Denmark), Sect. Sci., 8: 81. 1919.
- (5) SHERMAN, J. M., AND ALBUS, W. R. Jour. Bact., 3: 153. 1918.
- (6) SHERMAN, J. M., AND STARK, PAULINE. Jour. Bact., 22: 275. 1931.

IRRADIATED MILK: CHARACTERISTICS OF THE FLOWING FILM REQUIRED FOR OPTIMUM EFFICIENCY OF ANTIRACHITIC ACTIVATION

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Liquid films flowing over smooth surfaces are known to possess certain characteristics which conform to hydraulic principles and which may be designated as possessing smooth or turbulent flowing properties. Data have been obtained correlating the thickness of the milk film, speed of flow and volume of milk delivered per unit of time, with the antirachitic properties resulting from the application of ultra-violet rays of uniform intensity (1) (2). Even though a very large percentage of the radiations are absorbed by the first 0.02 millimeter depth of milk substance it was found that slowly moving films of this thickness were activated to a less degree per unit of time than faster flowing films of greater thickness. It is suggested that the character of the film may have been one of the factors determining these results. According to hydraulic principles the forward movement of each particle or molecule in films with true smooth flow characteristics is maintained, theoretically, in a single plane. In the case of films with turbulent flow characteristics this condition does not hold true, and in such films the particles or molecules, while maintaining the general direction of travel of the film as a whole, are, nevertheless, subject to influences tending to disturb the exact plane of travel, thus causing a degree of agitation or turbulence of the individual particles or molecules. It may be assumed therefore, that films with turbulent flow characteristics permit the exposure of a greater proportion of the milk substance at the air interface surface. Such a condition would seem to be highly desirable for the most effective and efficient antirachitic activation of milk. The data presented in this paper concerns the influence of film characteristics on the efficiency of utilization of the activating energy during the irradiation of milk.

EXPERIMENTAL

It was necessary to obtain data showing the relationship between the amount of milk delivered by given films per unit of time and the thickness of such films. The devices and methods previously described were used for determining the speed of flow, film thickness and distance traveled by

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the film during a given period (1). A curve showing the relationship of film thickness to the amount of milk delivered per unit of horizontal width per unit of time is shown in Chart I. Whole milk at a temperature of

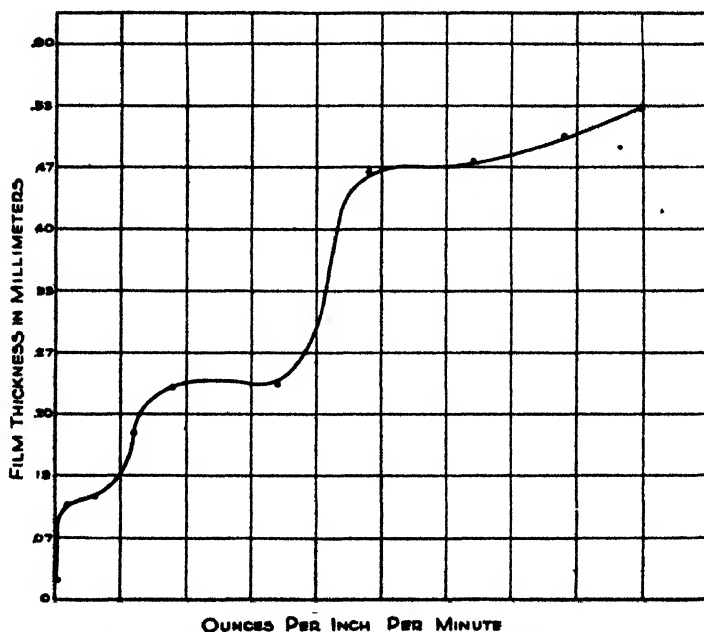


CHART I. THE RELATIONSHIP BETWEEN THE THICKNESS OF THE FLOWING MILK FILM AND FILM CAPACITY

65° F. and containing 3.6 per cent fat was used. The graph shows certain characteristics similar to those obtained from the study of other liquids wherein the transition between dominant smooth and turbulent flow characteristics is recognized by the breaks in the curve. The particular shape of this curve is probably due to the heterogeneous composition and physical characteristics of the milk. The curve indicates that smooth or turbulent flow characteristics are dominant at particular points throughout the range of conditions studied. The marked break in the curve between the film thickness of 0.23 millimeters and 0.46 millimeters is interpreted as the most significant transition range denoting the domination of smooth flow characteristics below 0.23 millimeters, in contradistinction to dominant turbulent flow characteristics in film thickness greater than 0.23 millimeters.

Having determined the relationship between film thickness and film capacity, certain films were duplicated and irradiated with ultra-violet rays of the same character and intensity employed in previous work (1) (2). The particular purpose of this investigation was to ascertain the relationship between film thickness, amount of milk delivered by the film per unit of time, film capacity and the vertical distance of travel necessary to give

a uniform degree of activation. The degree of activation which it was desired to obtain in the milk treated according to the specified conditions was the manifestation of a 2+ degree of calcification (3) (4) from 4 cc. of whole milk containing 3.6 per cent fat, fed daily during a period of 10 days following a 21 day period on the Steenbock rachitogenic diet No. 2965 (5). This degree of antirachitic potency has been shown by Hess and Lewis (6) (7) and Mitchell *et al.* (8) to be valuable in the prevention and cure of infantile rickets. The results are recorded in table 1 and plotted on logarythmic coordinates in Chart II.

TABLE 1

Characteristics of flowing milk films giving the same antirachitic potency when irradiated with the same intensity of ultra-violet radiations

NO.	FILM CAPACITY (OUNCES PER INCH PER MINUTE)	FILM THICKNESS	VERTICAL DISTANCE TRAVELED	EXPOSURE PERIOD
		<i>mm.</i>	<i>inches</i>	<i>secs.</i>
1	0.075	0.02	0.26	1.00
2	0.310	0.10	1.50	0.75
3	1.200	0.11	11.00	1.30
4	2.400	0.18	—	—
5	3.600	0.23	14.00	1.08
6	6.800	0.23	24.00	1.33
7	9.600	0.46	48.00	2.70
8	12.800	0.47	—	—
9	15.600	0.50	72.00	2.72
10	18.000	0.53	—	—

Inspection of the data, particularly Chart II showing the relationship between the distance of travel of the milk film and film capacity, indicates a definite correlation between film thickness and film capacity on the one hand, and the amount of vitamin D synthesized on the other. While other data have shown a general relationship of this character it is considered that the present results reveal more clearly the significance of the character of the flowing film as it is concerned in the power functions involved in the antirachitic activation of milk. The results are interpreted to mean that under the conditions of irradiation defined by the first part of the curve (Chart II) with uniform slope, the amount of energy applied is in excess of that which may be utilized most efficiently for activating the specified amount of milk to the particular degree selected as the criterion for these tests. The time necessary to bring about the specified degree of activation within the particular range of film thicknesses and film capacities may therefore, be considered as the irreducible minimum. Throughout the range of conditions represented by the flat portion of the curve the capacity of the film increases without substantial increase in film thickness. Since the

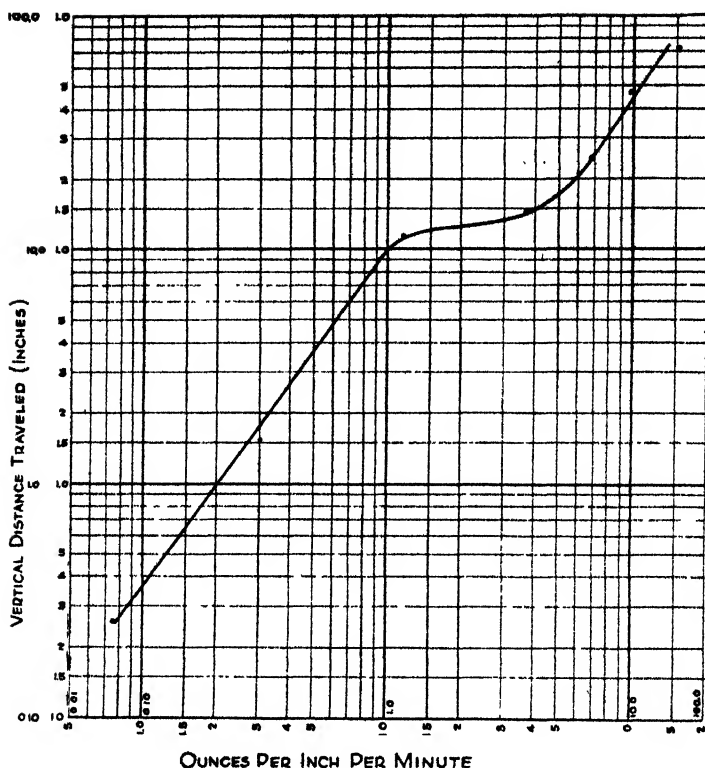


CHART II. THE RELATIONSHIP BETWEEN THE CAPACITY OF THE FLOWING MILK FILM AND DISTANCE OF TRAVEL NECESSARY TO GIVE A UNIFORM DEGREE OF ANTIRACHITIC ACTIVATION

degree of activation throughout this range is the same as for other conditions, the break in the curve is interpreted to mean that the applied energy is being used more efficiently than under any of the other conditions studied. It is believed that this greater efficiency in utilization of the applied radiations is caused primarily by the particular characteristics of the flowing film, whereby the correlation between vertical distance of travel, surface exposure of activable substances and film capacity are interrelated in a manner providing optimum conditions for activation within a minimum period of time. It is to be noted that in the upper part of the curve where a straight line of uniform slope has again been established by relatively thick films, which however, do not increase in thickness at a rate commensurate with the increase in film capacity, the time of exposure required to produce the specified degree of activation is about twice that required under optimum conditions. The films of higher capacities are believed to have predominately turbulent flow characteristics. However, from the standpoint of maintaining all conditions at their optimum, such films are less desirable

than the somewhat thinner films. Nevertheless the thick films are materially more desirable from a practical point of view than the very thin films of low capacity and less dominant turbulent flow characteristics.

CONCLUSIONS

1. The flow of milk films over smooth vertical surfaces by gravity can be differentiated as films with dominant smooth flow characteristics, or dominant turbulent flow characteristics.

2. Milk may be activated to a 2+ degree of calcification, as determined by standard assay procedures under a wide range of properly coordinated conditions involving film capacity, film thickness and distance of film travel, within a momentary period of exposure. There are certain optimum conditions wherein the applied energy is utilized more effectively and efficiently; such conditions are determined by the relationship between film thickness and film capacity.

3. Results indicate that the irreducible minimum in time necessary to give the specified degree of activation desired in these experiments was from 0.75 to 1.30 seconds. Exposure periods of 2.70 seconds gave the same degree of activation under conditions which permitted a marked increase in the amount of milk which could be activated per unit of time.

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REFERENCES

- (1) SUPPLE, G. C., AND DORCAS, M. J. Irradiated milk: The transmission and antirachitic activation of milk films by ultra-violet radiations. *JOUR. DAIRY SCI.* 17: 433. 1934.
- (2) SUPPLEE, G. C., FLANIGAN, G. E., BENDER, R. C., AND DORCAS, M. J. Irradiated milk: The influence of fat content and time of exposure on the antirachitic potency. *JOUR. DAIRY SCI.* 17: 483. 1934.
- (3) BILLS, C. E., AND McDONALD, F. C. Antirachitic substances: IV. The polymerization of cholesterol. *Jour. Biol. Chem.* 68: 821. 1926.
- (4) BILLS, C. E., HONEYWELL, E. M., WIRICK, A. M., AND NUSSMEIER, M. A critique of the line test for vitamin D. *Jour. Biol. Chem.* 90: 619. 1931.
- (5) STEENBOCK, H., AND BLACK, A. Fat soluble vitamins: XXIII. The induction of growth-promoting properties in fats and their unsaponifiable constituents by exposure to light. *Jour. Biol. Chem.* 64: 263. 1925.
- (6) HESS, A. F., AND LEWIS, J. M. Milk irradiated by the carbon arc lamp: A clinical and laboratory study of rickets. *Jour. Am. Med. Assoc.* 99: 647. 1932.
- (7) HESS, A. F., AND LEWIS, J. M. An appraisal of antirachitics in terms of rat and clinical units. *Jour. Am. Med. Assoc.* 101: 181. 1933.
- (8) MITCHELL, J. McK., EIMAN, J., WHIPPLE, D. V., AND STOKES, J., JR. The protective value for infants of various types of vitamin D fortified milk. *Am. Jour. Pub. Health* 22: 1220. 1932.

SOME PHYSICO-CHEMICAL PROPERTIES OF LACTOSE

II. FACTORS INFLUENCING THE CRYSTALLINE HABIT OF LACTOSE

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INTRODUCTION

Photographs, or sketches of lactose crystals have been published by a number of men. All are assumed to be alpha hydrate, yet on casual inspection, some of the crystals seem quite different from others. That lactose solutions do yield crystals of quite different appearance, depending upon conditions of crystallization, may be confirmed by microscopic examination. This fact seemed worthy of further investigation for several reasons. It seemed possible that some of the strange crystals might not be alpha hydrate at all. Beta hydrate has never been isolated and recognized, nevertheless, it might appear as an unstable phase under certain conditions; and since a molecular compound of alpha and beta lactose has been prepared (5), others might exist.

The first problem, therefore, was to establish the identity of the various crystals, and to determine the relation between the different forms. If all proved to be alpha hydrate, then the second problem would be to determine what factor, or factors, is responsible for such striking differences in crystal habit. If the factors responsible for the appearance of different forms were known, then it might be possible to examine the crystals in such products as sweetened condensed milk, or sandy ice cream, and learn something about the conditions under which the crystals were formed.

Hunziker and Nisson (6, 7) reported that sucrose modified the form of lactose crystals. Williams and Peter (15) reported a similar observation. It is well known that the crystalline habit of many substances may be altered by the presence of other compounds. Saylor (11) has given a good discussion of the theory of this phenomenon. It is due to selective adsorption on certain faces of the crystal. The possibility of such an adsorption of sucrose by lactose seemed worthy of investigation because of its application to the crystallization of lactose in ice cream and in sweetened condensed milk.

In order to answer some of these questions, crystals of lactose were allowed to form under various conditions, and in the presence of various added substances. The shape of these crystals was studied carefully. The

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relation between the different crystalline types was determined as far as possible, and an attempt was made to correlate the type of crystal with the conditions under which crystallization took place. This paper is a summary of the observations which were made during these investigations.

EXPERIMENTS

Crystals were prepared for examination by placing drops of supersaturated lactose solution upon microscope slides and then protecting them from evaporation by means of cover slips sealed down with a mixture of vaseline and beeswax. These slides were held at a constant temperature for several days before examination. Photographs of several well developed crystals of alpha hydrate prepared in this manner are shown in figure 3.

The diagram of a lactose crystal given by Groth (4) shows ten faces. This diagram is also given by Hunziker and Nisson (7) who state that the fully developed lactose crystal has ten faces. However, if crystals are grown very carefully, at least sixteen faces may be found. Some of the crystals shown in figure 3 have thirteen faces. In their own paper, Hunziker and Nisson published photographs showing faces not included among the ten. Crystals having more than 13 faces are extremely rare, but the new faces are of interest because they appear on some crystal types of frequent occurrence.

In addition to studying crystals grown on microscope slides, examinations were made of crystals grown in test-tubes, in ice cream, in sweetened condensed milk, in agar and in gelatine jellies, and in other ways. Figures 2 and 3 show some of the characteristic crystal types which were found. All of these crystals proved to be alpha hydrate.

The relationship of the different forms to one another might be shown by either of two methods: first, by studying a large number of crystals and tracing the gradual transition from one type to another; and second, by so altering conditions during crystallization that one form of crystal would change over to another form while the crystal was under constant observation. Both methods were used in this study.

Although Sato (10), and Hunziker and Nisson (6, 7), described pyramidal shape as being characteristic of lactose crystals grown in sucrose solutions, it was found that pyramidal crystals may be grown in the absence of sucrose, and that normal lactose crystals may be grown in the presence of large amounts of sucrose. Cane sugar has no specific action upon the crystalline habit of lactose. A study of crystals produced by cooling, by evaporation, and by precipitation by alcohol, led to the conclusion that the rate of growth, or what might be termed the "crystallization pressure," was the principal factor determining the form of the crystals.

When the crystallization of lactose is forced rapidly enough, only prisms are formed. As the precipitation pressure becomes less the prisms become

shorter and broader. With further decrease, the crystals appear first as diamond-shaped plates. These have been observed by Williams and Peter (15). These diamonds form the base of the pyramidal crystals of Sato, and of Hunziker and Nisson. At slightly lower degrees of supersaturation, these plates grow in thickness forming pyramids. Crystals appearing in more dilute solutions lie on a different face, and appear as triangular plates having two sides equal. This is the outline of the ordinary "tomahawk." As crystallization proceeds slowly, the crystals increase in thickness, and bevel faces appear on the short side of the triangle, thus sharpening the blade of the tomahawk. At a later stage, it becomes apparent that the apex of the triangle is not a point, or a line, but a surface. Still later, another pair of bevel faces appear, completing the set of ten faces described by most authors. Figure 1 shows sketches illustrating this gradual transition. Photographs of these various forms have already been referred to in figure 2.

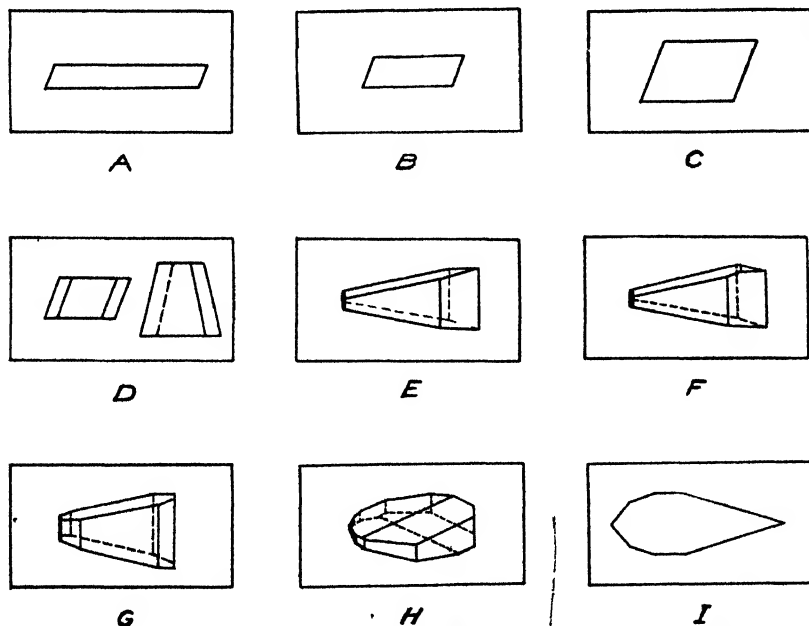


FIG. 1. THE CRYSTALLINE HABIT OF LACTOSE ALPHA HYDRATE

- A. Prism, formed when velocity of growth is very high.
- B. Prism, formed more slowly than prism A.
- C. Diamond shaped plates, transition between prism and pyramid.
- D. Pyramids resulting from increase in thickness of diamond.
- E. Tomahawk, a tall pyramid with bevel faces at the base.
- F. Tomahawk, showing another face which sometimes appears.
- G. The form most commonly described as "fully developed."
- H. A crystal having 13 faces. The face shown in F is not present.
- I. A profile view of H with the tomahawk blade sharpened.

If crystallization takes place very slowly, other faces may be observed. The small surface at the apex of the crystal becomes two surfaces. These faces may be found in some of the photographs published by Hunziker and Nisson (7). Later two additional faces may appear running obliquely along the crystals. Their position is shown in figures 1-H and 3-A. Finally, another pair of parallel faces may appear cutting off the corners of the hatchet blade, figures 1-F and 3-E.

Different combinations of these many faces produce a great variety of crystal forms which, at first glance, seem to bear no resemblance to each other. However, if the fundamental relationship between the faces is understood, such crystals may usually be recognized without difficulty.

The relationship between the different crystal types illustrated in figures 1, 2 and 3 may be traced out by the examination of a large number of crystals showing the transition from one form to the other by almost imperceptible degrees. This relationship may also be shown more directly by observing changes in the growth of a single crystal when the conditions of crystallization are altered. Tomahawk crystals can be formed in a drop of lactose solution on a microscope slide, and then, when evaporation is hastened, by means of a current of air, the tomahawks will grow in width becoming "broad axes" or even prisms. This is illustrated in figure 2-H.

The reverse phenomenon may also be observed, lactose precipitated quickly by alcohol separates first in the form of prisms, but as crystallization slows down, the prisms become wider and tend to revert toward the "broad axe" or the tomahawk form, figure 2-G.

An examination of lactose crystals indicates that they have only a single axis of symmetry. This has been confirmed by Elings and Terpstra (2), and it offers an explanation for a phenomenon that was puzzling. When dehydrated lactose is removed from the drying oven for weighing, the crystals adhere together resembling wet sand. Alpha anhydride has a great affinity for water, and it seemed that traces of moisture were responsible for this effect. However, all precautions failed to prevent this apparent absorption of moisture by such samples before weighing, and it was finally discovered that the phenomenon was due to an entirely different cause. Crystals possessing only an axis of symmetry become electrically charged at the poles when they experience a change in temperature (12, also 13, volume 2, p. 1393). These charges are responsible for the coherence of the lactose crystals, for their apparent dampness after their removal from a drying oven. Partially dehydrated lactose in an evacuated and sealed tube exhibited this phenomenon, either when warmed over a flame, or when chilled in a freezing mixture, proving that surface moisture was not the cause of the apparent dampness. Since completely dehydrated alpha lactose shows the same behavior, it appears that alpha anhydride crystals must also possess only one axis of symmetry.

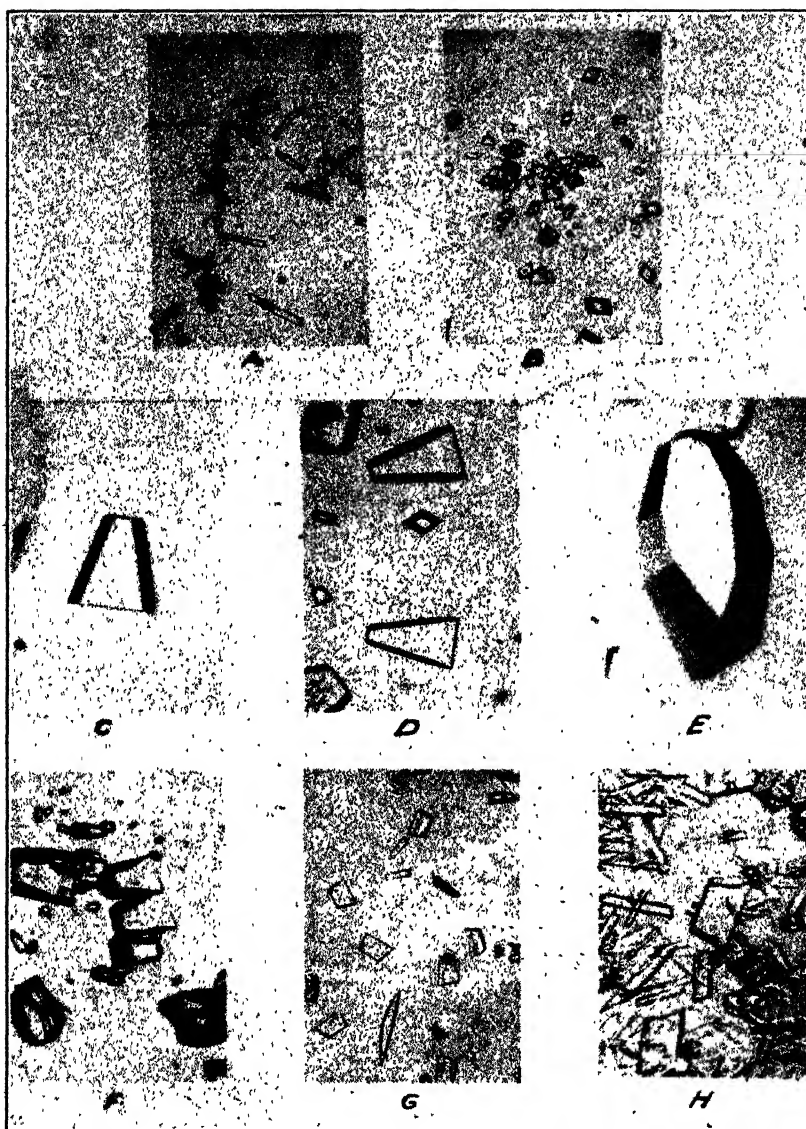


FIG. 2. PHOTOGRAPHS OF LACTOSE CRYSTALS

- A. Prism form. Compare with Figure 1-A.
- B. Diamond form. Compare with Figures 1-C and 1-D.
- C. Pyramids. Compare with Figure 1-D.
- D. Tomahawk form. Compare with Figure 1-E.
- E. The form usually described as "fully developed."
- F. The broadaxe form. A shortened tomahawk.
- G. Lactose prisms transferred to a solution only slightly supersaturated with lactose. The prisms are reverting to the tomahawk form through the broadaxe stage.
- H. Crystals formed by the evaporation of a drop of lactose solution. A few seconds earlier, the large crystal in the center was a well formed tomahawk. Rapid evaporation is resulting in the formation of prisms.

The formation of crystal clusters takes place more readily when the precipitation pressure is high, but the effect of precipitation pressure upon cluster formation is not as clear cut as its effect upon the form of the crystals. Other factors must influence cluster formation because the clusters often develop when the degree of supersaturation is very low. In spite of the greatest care, it has not been possible to grow lactose crystals to sizes greater than a half gram without having branch crystals appear on the surfaces.

One observation regarding cluster formation seems worthy of mention. Whenever a new crystal appears as an outgrowth of another, it always has the blade of the tomahawk directed away from the parent crystal. The apex of the second crystal is always directed toward the first. No exception to this rule was ever found although more than a hundred cases were examined. This seems the more remarkable since the second crystal may appear at any point upon the surface of the first (although they usually appear near the apex) and since there seems to be no definite orientation of the axes of the two crystals.

Mention has already been made of the fact that sucrose is said to cause lactose to crystallize as pyramids. While such crystals are often formed in the presence of sucrose, the sucrose has no specific action upon the crystallization of lactose. Highly developed crystals of lactose may be grown in concentrated sucrose solutions, and pyramidal crystals may be grown from solutions of lactose in pure water. What influence sucrose may have is probably due to its influence upon the rate of crystallization, by increasing the viscosity, and by lowering the solubility of lactose. Peter (9) found that the solubility of lactose at 0° C. was reduced nearly one-half by saturating its solutions with sucrose.

There has been considerable debate, by a number of workers, concerning the effect of foreign substances upon the crystallization of lactose. Leighton and Peter (8) and Fujimoto (3) have studied the effect of dyes. No significant action was found. There seems no reason why dyes should influence crystallization unless they are adsorbed by the crystals. Such an adsorption of dyes should easily be detected if it occurred, but neither author mentions any staining of the crystals. Fujimoto believed that both gelatine and sucrose inhibited the crystallization of lactose. On the other hand, Dahle (1) believed that other sugars had no influence upon the crystallization of lactose, and that gelatine had practically no effect. Dahle also reported that rennet coagulation had no influence. Hunziker and Nisson (6) reported that the colloids of milk had no influence upon either the crystal form of lactose, or upon its solubility.

It is not always easy to distinguish between the specific effect of added substances, and the effect of crystallization velocity, upon the crystalline form of lactose. Most studies on the crystallization of lactose have failed

to distinguish between the rates of nuclei formation and the rates of crystal growth. The rate of nuclei formation may be influenced by many factors, and most of those who have studied the crystallization of lactose in ice cream have not taken this into account. Moreover, experiments on the rate of crystal growth have little value unless the number of nuclei present is controlled. For these reasons, it is not surprising that there should be such disagreement regarding the effect of foreign substances upon lactose crystallization.

Some experiments were carried out to determine the effect of a few substances upon the crystallization of lactose. In order to exaggerate any possible action, the foreign substance was added in relatively large amounts. Thirty-five grams of dextrin, of sucrose, or of gum arabic, added to 100 cc. of a solution of lactose saturated at 60° C., seemed to have no definite influence upon the crystallization of lactose. A similar quantity of gelatin caused the formation of dense clusters of crystals resembling those formed by an aqueous solution of lactose saturated at 80° C., when it was allowed to crystallize at 0° C. Starch seemed to act similarly but observation was practically impossible because of the opacity. Molar concentrations of urea, of ammonium chloride, of sodium acetate, and of potassium chloride seemed to have no specific effect upon crystallization. The action of salts, however, will be considered again in papers five and six. These results are not to be considered as conclusive evidence that the substances mentioned have no effect. However, in dilute solution any action which they may have will be slight. On the other hand, preliminary experiments have indicated that beta lactose does retard the crystallization of alpha hydrate. However, this phenomenon must be investigated more thoroughly before it is finally accepted.

The solubility of lactose is greatly reduced by the presence of alcohol. Additions of alcohol, therefore, accelerate the crystallization of lactose and thus indirectly influence the crystal habit to a marked degree. The addition of alcohol to cold saturated solutions of lactose results in another interesting phenomenon. When the alcohol is added to the sugar solution, the mixture becomes milky white for a few seconds, and then clears up again. After a few minutes, a permanent precipitate of lactose crystals will appear. Microscopic examination proved that the formation of an emulsion was responsible for the transitory milkiness. This is of interest because alcohol and water are miscible in all proportions. Presumably, some solid phase is precipitated in the interface, but its nature is as yet unknown. If it were alpha hydrate, there seems no reason why it should apparently dissolve again, yet often there is a lag of several minutes between the time of clearing and the time when the visible crystallization of lactose takes place.

Wherry (14) has published crystallographic data regarding beta lac-

tose. No attempt was made to study in detail the variation of the crystalline habit of this sugar with changes in conditions. However, it was observed that beta lactose also forms prisms when crystallization is sufficiently rapid. The prisms of beta lactose may be distinguished from those of alpha hydrate by the fact that they are curved, figure 3-H. They may be prepared by dissolving beta lactose in ice-water and then precipitating with alcohol, or by the crystallization of such a solution at low temperatures by rapid evaporation. The curved needles may also be found in the precipitate formed by adding alcohol to equilibrium solutions of lactose.

SUMMARY

The crystalline habit of alpha hydrate varies greatly under different condition of crystallization.

The relationship between the different types of crystals was traced: first, by examining many crystals varying by imperceptible degrees from one another; and second, by changing conditions during crystallization, thereby causing crystals to develop new faces and to alter their habit of growth.

The principal factor governing the crystalline habit of lactose is the precipitation pressure, the ratio of the actual concentration to the solubility. By varying this ratio, a great variety of crystal types may be produced.

The influence of sucrose upon the crystalline form of lactose is not a specific action but is due to its precipitating effect upon that sugar.

Both alpha hydrate and beta anhydride will form needles if crystallization is sufficiently rapid. The two may be distinguished readily by the fact that the prisms of alpha hydrate are always straight while those of beta anhydride are curved.

REFERENCES

- (1) DAHLE, CHESTER D. Sandy ice cream. *Proceedings World's Dairy Congress* 1: 500-10. 1924.
- (2) ELINGS, S. B., AND TERPSTRA, P. Prüfung einiger Krystallarten auf Piezoelektrizität. *Z. Kryst.* 67: 279-84. 1928.
- (3) FUJIMOTO, A. T. Some factors influencing the rate of crystallization of lactose in ice cream. M. S. Thesis. Ohio State University. 1926.
- (4) GROTH, P. H. VON. *Chemische Krystallographie*, vol. 3, p. 450. Wilhelm Engelmann, Leipzig. 1910.
- (5) HUDSON, C. S., AND HOCKETT, R. C. A novel modification of lactose. *Jour. Am. Chem. Soc.* 53: 4455-56. 1931.
- (6) HUNZIKER, O. F., AND NISSON, B. H. Lactose solubility and lactose crystal formation. I. Lactose solubility. *JOUR. DAIRY SCI.* 9: 517-37. 1926.
- (7) HUNZIKER, O. F., AND NISSON, B. H. Lactose solubility and lactose crystal formation. II. Lactose crystal formation. *JOUR. DAIRY SCI.* 10: 139-54. 1927.
- (8) LEIGHTON, A., AND PETER, P. N. Factors influencing the crystallization of lactose. *Proceedings World's Dairy Congress* 1: 477-85. 1924.
- (9) PETER, PHILIP N. Solubility relationships of lactose-sucrose solutions. I. Lac-

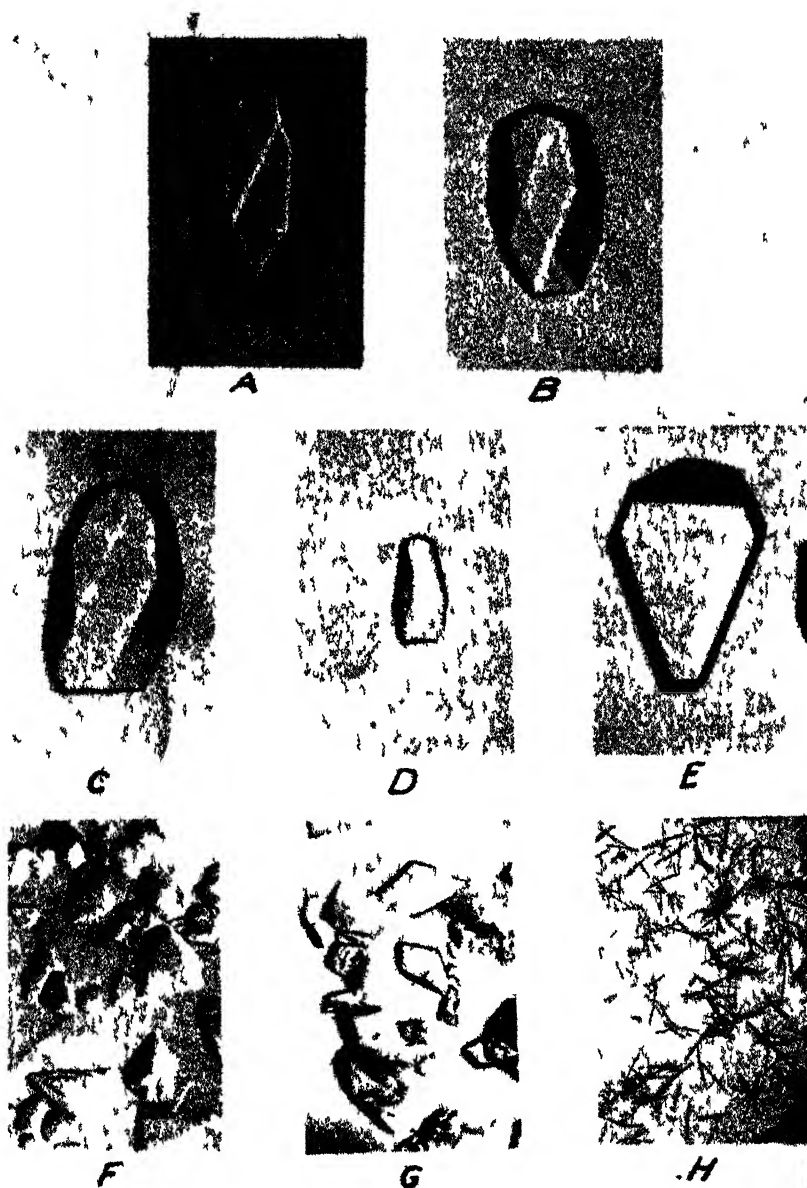


FIG. 3. PHOTOGRAPHS OF LACTOSE CRYSTALS

- A, B, C, and D. Lactose crystals having 13 faces. Compare with Figure 1-II.
 E. A lactose crystal lying on the face shown in Figure 1-F. Here it is a major face.
 Compare also with Figure 1-I.
 F. Crystals found in a commercial preparation of lactose alpha hydrate.
 G. Crystals found in a commercial preparation of beta lactose.
 H. Curved needle crystals of beta lactose.

- tose-sucrose solubilities at low temperature. *Jour. Phys. Chem.* **32**: 1856-64. 1928.
- (10) SATO, M. The crystals found in sweetened condensed milk. *Proceedings World's Dairy Congress* **2**: 1285. 1923.
- (11) SAYLOR, CHARLES HAMILTON. Calcite and aragonite. *Jour. Phys. Chem.* **32**: 1441-60. 1928.
- (12) SPENCER, L. J. Pyroelectrical phenomena of crystals. *Encyclopedia Britannica* **6**: 828 (14th edition). 1929.
- (13) TUTTON, A. E. H. *Crystallography and Practical Crystal Measurement*. McMillan and Company, London. 1922.
- (14) WHERRY, EDGAR T. The crystallography and optical properties of beta lactose. *Jour. Wash. Acad. Sci.* **18**: 302-4. 1928.
- (15) WILLIAMS, O. E., AND PETER, P. N. A new form of lactose crystal found in sandy ice cream. *JOUR. DAIRY SCI.* **13**: 471-7. 1930.

THE RELATION BETWEEN THE HARDNESS OF BUTTER AND BUTTERFAT AND THE IODINE NUMBER OF THE BUTTERFAT*

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Considerable data have been secured at the University of Minnesota concerning the relation between the chemical constants of butterfat and the hardness of the butterfat and butter. This has been one of the problems encountered in studies of the effect of various feeds on the character of butterfat.

METHOD OF DETERMINING HARDNESS

Numerous methods have been used to estimate the hardness of butter and butterfat. The method most commonly employed has been to measure the depth of penetration of rods of known weight into the material to be tested. One of the best examples of the apparatus employed for this method is that of Perkins (1) who reviews the more important literature on the subject prior to 1914.

The values herein reported for the hardness of the butter and butterfat were determined by the use of an apparatus similar to that used by Templeton and Sommer (2) for measuring the body of cheese. It was found necessary to use cubes of a different size than those used by Templeton and Sommer, and a special technique in their preparation. The hardness of the butter or butterfat was measured by determining the force required to crush a three-fourths inch cube to two-thirds of its original thickness. The weight (in grams) of mercury required was used as a measure of the relative hardness of the sample. The actual force required to crush the cube was, however, five times that indicated by the grams of mercury since the pulley-arrangement gave a mechanical advantage of 5.

The cubes of butter or butterfat were prepared in brass molds of the proper size. The butter was carefully tamped into the molds immediately after working. The molds were placed in the refrigerator at -18° C. for 24 hours, after which the cubes were removed from the molds, and tempered in a water bath maintained at 12° C. (± 0.25) for 24 hours before being used for the hardness determination.

The cubes of butterfat were prepared using the same molds. The molds were placed on trays and set at -18° C. for a sufficient period of time to be

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thoroughly chilled. The melted butterfat at about 37° C. was poured into the molds and tempered in the same way as the butter cubes before being used for the hardness determination. Six cubes were prepared for each sample. The hardness values reported are an average of the determinations made on the six cubes.

Accuracy of the hardness determination: The force required to crush different cubes of the same sample of butter or butterfat varied appreciably due probably to mechanical defects in the cubes. Maximum deviation from the mean, however, rarely exceed 5 per cent for individual cubes. Satisfactory agreement was consistently secured on determinations made on separate churnings of the same cream. Representative values are shown in table 1.

TABLE 1
Comparative hardness of butter and butterfat from different churnings of the same lot of cream

CHURNING	HARDNESS OF BUTTER	HARDNESS OF FAT
<i>number</i>	<i>grams</i>	<i>grams</i>
1	574	1250
2	578	1303
3	549	1255
4	572	1223
5	573	1218
6	542	1203
7	579	1193
8	558	1246
Mean	566	1236
Standard deviation	13.1	32.7

ORIGIN OF BUTTER SAMPLES

The butter samples were churned from cream secured from cows in the University herd. The cream was separated from the milk of individual cows for 199 churnings and from the milk of groups of cows for 104 churnings. A standard¹ churning and working procedure was used for those samples on which values are reported for the hardness of butter in order

¹ The cream after separation was cooled, standardized with skim milk to 30 per cent butterfat and held at 5° C. for 5 hours. A battery of two gallon glass churns of the "Dazey" type was used in an arrangement similar to that devised by Guthrie and Sharp (3). Six pounds of cream were weighed into the churns and the whole held in the water bath at 11° C. for one hour before churning. The churning temperature used was 11° C. The butter granules were washed once with a volume of water equal to the volume of buttermilk at 11° C. All free moisture was allowed to drain from the granules and the butter worked with a wooden butter ladle on a board arranged in such a fashion that the surplus water could readily drain away. The butter was worked until the moisture was judged to be thoroughly incorporated.

to eliminate in so far as possible the effect of variations in the churning and working conditions on the hardness of the butter.

RELATION BETWEEN HARDNESS OF BUTTER AND HARDNESS OF BUTTERFAT

The assumption is logical that the hardness of butter should be dependent to a large extent upon the hardness of the butterfat. No experimental evidence is available, however, in support of this assumption. Perkins (1) found no direct relation between the hardness of the butter and the hardness of the butterfat among 3 samples examined. Unquestionably, factors other than the hardness of the butterfat may influence the hardness of the butter. These may reasonably include the moisture content, the distribution of the moisture, and the air content of the butter, as well as variations in the churning and working conditions. For example, Perkins secured marked differences by varying the working conditions. Haglund, Wode, and Olsson (4) and Wode (5) were able to produce permanent variations by changes in the manufacturing methods.

Since the hardness of butter is affected by factors other than the character of the butterfat, it was considered desirable to relate variations in the composition of the butterfat to the hardness of the butterfat rather than to the hardness of the butter. The influence of variations in the composition of butterfat on the hardness of butter can then be interpreted from the relation between the hardness of the butter and the butterfat.

Hardness determinations were made on 85 samples of butter and on the butterfat rendered from the butter. The relationship between the two variables is shown in figure 1. It is evident from the chart that the hardness of these samples of butter, made under uniform conditions, is closely dependent upon the hardness of the butterfat. The correlation coefficient

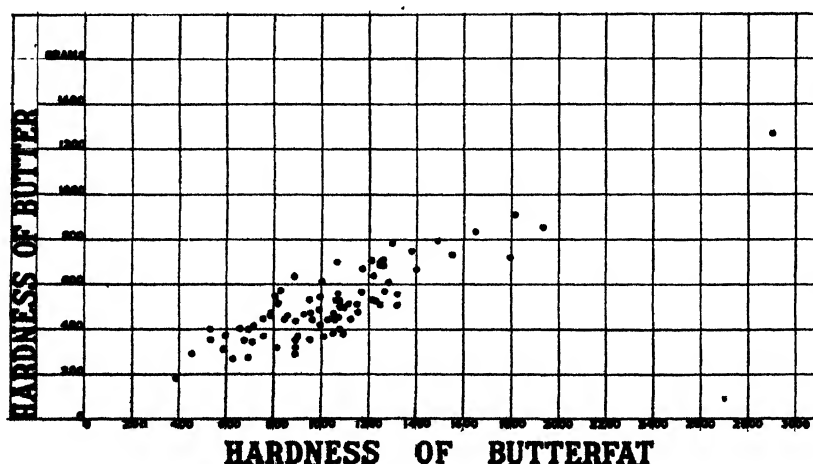


FIG. 1. RELATION BETWEEN THE HARDNESS OF BUTTER AND THE HARDNESS OF BUTTERFAT.

between the two sets of values is +0.87 with a P value (Fisher (6)) of less than 0.01. The correlation is thus highly significant. The hardness of the butter (x_1) may be estimated from the hardness of the butterfat (x_2) by the regression equation $x_1 = 0.42 x_2 + 80.7$ with a standard error of estimate of 82 grams. The relationship between the two variables appears to be strictly linear.

RELATION BETWEEN THE HARDNESS OF BUTTER AND MOISTURE CONTENT OF BUTTER

Though the hardness of the butter is thus closely dependent upon the hardness of the butterfat, considerable variation in the hardness of the butter samples is unaccounted for. The moisture content of the butter samples ranged from 11.0 to 26.6 per cent. Variations of this magnitude should influence the hardness. The inclusion of the moisture content of the butter (x_3) as an independent variable influencing the hardness of the butter results in the regression equation, $x_1 = 0.382 x_2 - 12.07 x_3 + 285.6$. From this equation the hardness of the butter may be estimated with a standard error of estimate of 77 grams. The multiple correlation coefficient is 0.89.

Further proof that variations in the moisture content of the butter slightly, though significantly, influence its hardness is evidenced by the partial correlation coefficients. The partial correlation coefficient between the hardness of the butter and the moisture content of the butter with the hardness of the butterfat held constant is -0.34, with a P value of less than 0.01. The partial correlation coefficient between the hardness of the butter and the hardness of the butterfat with the moisture content of the butter held constant is +0.86.

RELATION BETWEEN IODINE NUMBER OF BUTTERFAT AND MOISTURE CONTENT OF THE BUTTER

In view of the standard churning and working procedure which was used, the marked variations in the moisture content of the butter must be related to variations in the character of the butterfat. Hunziker and associates (7) first pointed out that the unsaturated fatty acids in the butterfat have an influence on the moisture-retaining properties of the butter. Palmer (8) suggests that a probable explanation of this fact is found in the work of Langmuir, and of Harkins and associates, showing that it is the unsaturated fatty acids which increase the water-covering capacity of a fat. In the present data, there is a direct relationship between the iodine number of the butterfat and the moisture content of the butter. This is shown in figure 2 in which the moisture content of the butter samples is shown plotted as ordinates and the iodine number of the butterfat as abscissas. It is plainly evident that the moisture content of the butter increases with an

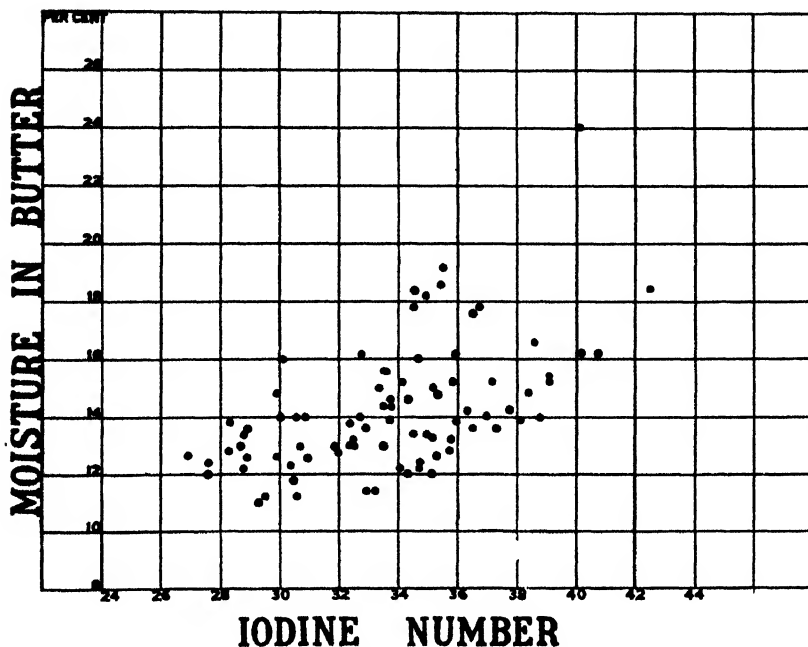


FIG. 2. RELATION BETWEEN THE IODINE NUMBER OF BUTTERFAT AND THE MOISTURE CONTENT OF THE BUTTER.

increase in the iodine number of the butterfat. The correlation coefficient between the two sets of values is +0.55 with a P value of less than 0.01. One sample with a moisture content of 26.6 per cent has not been included in this comparison, since the sample was too small to permit working the butter by the standard procedure.

RELATION BETWEEN FAT CONSTANTS OF BUTTERFAT AND ITS HARDNESS

Several investigators, notably Haglund, Wode, and Olsson (4), have shown that the hardness of butter is inversely proportional to the iodine number of the butterfat. These workers used the Perkins method for determining the hardness of 413 samples of creamery butter. The iodine number of the butterfat and hardness were plotted and fitted to a third degree parabola. The equation found for the curve was $x = -2.04 - 0.572y + 0.0351y^2 - 0.000734y^3$. The correlation coefficient was 0.922 ± 0.007 . These values indicate a close relation between iodine value and hardness of butter. Variations in the hardness of samples with the same iodine values were ascribed, by these workers, to differences in the manufacturing process.

Figure 3 shows the relationship between the hardness of butterfat and the iodine value for the 303 samples² on which data were secured in this

² Data for 59 samples from an experiment supervised by Dr. W. E. Petersen are included in this analysis.

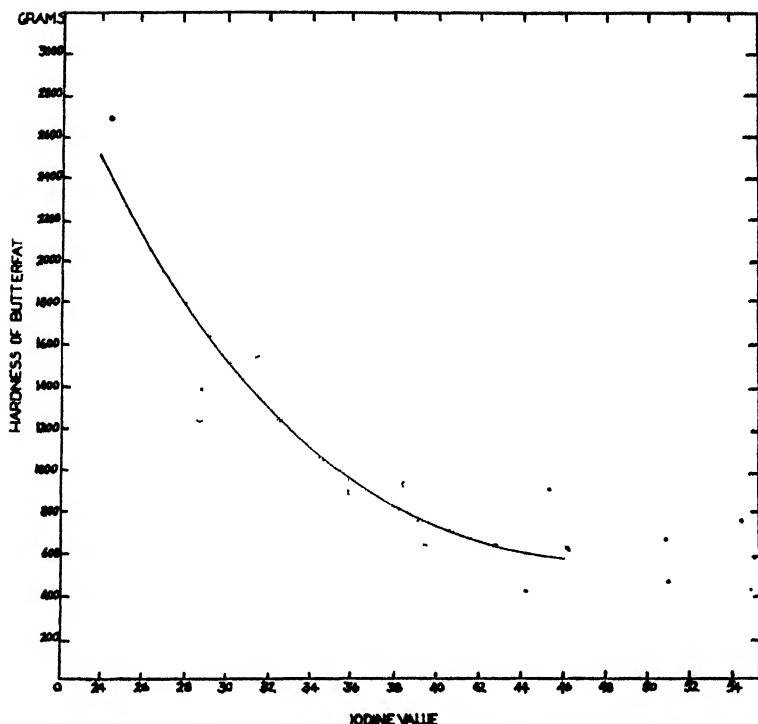


FIG. 3. RELATION BETWEEN THE IODINE NUMBER AND THE HARDNESS OF BUTTERFAT.

work. The hardness values are plotted as ordinates and the iodine numbers as abscissas. The relationship between the two variables, as seen from the chart, is definitely curvilinear. The method followed in working out the correlation index was that given by Ezekiel (9). The observations were grouped and averaged and a free-hand curve drawn through the mean values as smoothly as possible. This curve established the estimated value for the hardness of butterfat corresponding to a given iodine value. The correlation index determined in this way between the two sets of values is 0.85, which has a *P* value of less than 0.01. The standard error of estimate is 238.5 grams. This error of estimate represents 53 per cent of the standard deviation of the original values for hardness of butterfat.

Haglund, Wode, and Olsson (4) attribute deviations from the regression curve of hardness of butter as determined by iodine value to variations in the method of manufacture. This cannot be a factor when dealing with hardness of butterfat. The Reichert-Meissl number of the butterfat was determined as well as the iodine number. The inclusion of the Reichert-Meissl number in a multiple correlation indicated that considering the data as a whole, variations in the Reichert-Meissl number did not significantly influence the hardness of the butterfat. Extreme variations in the

Reichert-Meissl number may, however, be associated with considerable changes in the hardness of the butterfat. This can be shown from the data for individual cows in which there were extreme variations in the Reichert-Meissl number. This is shown in figure 4 in which the deviations from the

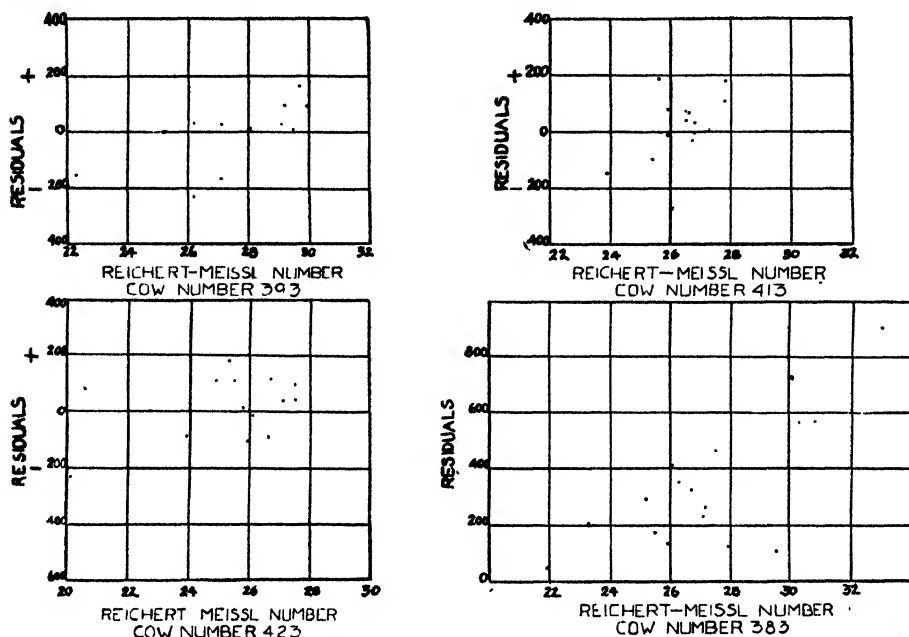


FIG. 4. RELATION BETWEEN THE REICHERT-MEISSL NUMBER AND THE HARDNESS OF BUTTERFAT.

regression curve are plotted as ordinates and the values for the Reichert-Meissl number as abscissas. The data for cows 383 and 423 show a definite decrease in the hardness of butterfat with an increase in the Reichert-Meissl number. On the other hand the data from cow 393 and especially from cow 413 do not show this relationship. This is probably due to the comparatively narrow range of the Reichert-Meissl values for the fat from these animals. It is evident from chart 4 that when the Reichert-Meissl values range from 25 to 28 the hardness of the butterfat may vary without regard to the Reichert-Meissl number. Variations in the Reichert-Meissl number beyond this range appear to be correlated with the hardness.

Factors such as variations in the fatty acid composition of the butterfat other than those revealed by the iodine number and the Reichert-Meissl number, or variations in the glyceride structure of the butterfat, must be responsible for most of the deviations from the regression curve of the data herein reported.

A noticeable difference was observed in the deviation from the regression curve of samples from the various breeds. The mean deviations were as follows:

<i>Breed</i>	<i>Number of Samples</i>	<i>Mean Deviation</i>
Ayrshire	19	+ 69
Holstein	129	+ 107
Channel Island	51	- 69

Thus it is apparent that the Channel Island breeds produce butterfat which for a given iodine value is firmer than that produced by Holsteins or Ayrshires.

SUMMARY AND CONCLUSIONS

The method of Templeton and Sommer for measuring the body of cheese was found adaptable for determining the hardness of butter or butterfat. When a standard churning and working procedure is used, the hardness of butter is directly proportional to the hardness of the butterfat. Variations in the moisture content of the butter slightly influence its hardness. The moisture content of butter, where a standard churning and working procedure is used, increases with an increase in the iodine number of the butterfat.

There is a highly significant correlation between the hardness of the butterfat and the iodine number of the butterfat. Extreme variations in the Reichert-Meissl number of the butterfat may be associated with variations in the hardness of the butterfat. Butterfat from cows of the Jersey or Guernsey breeds is somewhat firmer than butterfat with the same iodine value from cows of the Holstein or Ayrshire breeds.

REFERENCES

- (1) PERKINS, A. E. An apparatus and method for determining the hardness of butterfat. *Ind. and Eng. Chem.* 6: 136-141. 1914.
- (2) TEMPLETON, H. L., AND SOMMER, H. H. Some observations on process cheese. *JOUR. DAIRY SCI.* 13: 203-220. 1930.
- (3) GUTHRIE, E. S., AND SHARP, F. P. The effect of hydrogen-ion concentration on the churning time of cream. *JOUR. DAIRY SCI.* 14: 1-13. 1931.
- (4) HAGLUND, E., WODE, G., AND OLSSON, T. Undersökningar över det Svenska smörets konsistens. Meddel. 387. Centralanst. Försoksv. Jordbruksområdet (Sweden). 1930.
- (5) WODE, G. Undersökningar över det Svenska smörets konsistens. II Olika faktorerers inflytande på smörets hårdhet. Meddel. 438. Centralanst. Försoksv. Jordbruksområdet (Sweden). 1933.
- (6) FISHER, R. A. Statistical methods for research workers. Oliver and Boyd. London, pp. 159-61. 1930.
- (7) HUNZIKER, O. F., MILLS, H. C., AND SPITZER, G. The moisture control of butter. I. Factors not under control of the buttermaker. *Purdue Agr. Expt. Sta. Bul.* 159: 285-360. 1912.
- (8) PALMER, L. S. The chemistry of milk and dairy products viewed from a colloidal standpoint. *Ind. and Eng. Chem.* 16: 631-642. 1924.
- (9) EZEKIEL, MORDECAI. Methods of correlation analysis. John Wiley and Sons, New York, pp. 130-134. 1930.

THE RELATION OF THE COMPOSITION OF BUTTERFAT TO THE CHURNABILITY OF CREAM*

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The Division of Dairy Husbandry of the University of Minnesota has been interested for several years in controlling the fat losses in buttermilk when churning sweet cream. At the Minnesota State Experimental creamery, which was operated under the supervision of the University, an effort was made to reduce such fat losses to a minimum. This work was started in 1926. After that date observations were made on 3800 churnings. The data, on which this report is based, have accumulated since 1927, when it was observed that the monthly average fat content of the buttermilk fluctuated widely and without exhibiting any definite trends. By systematic checking of the butterfat losses and by giving careful attention to churning

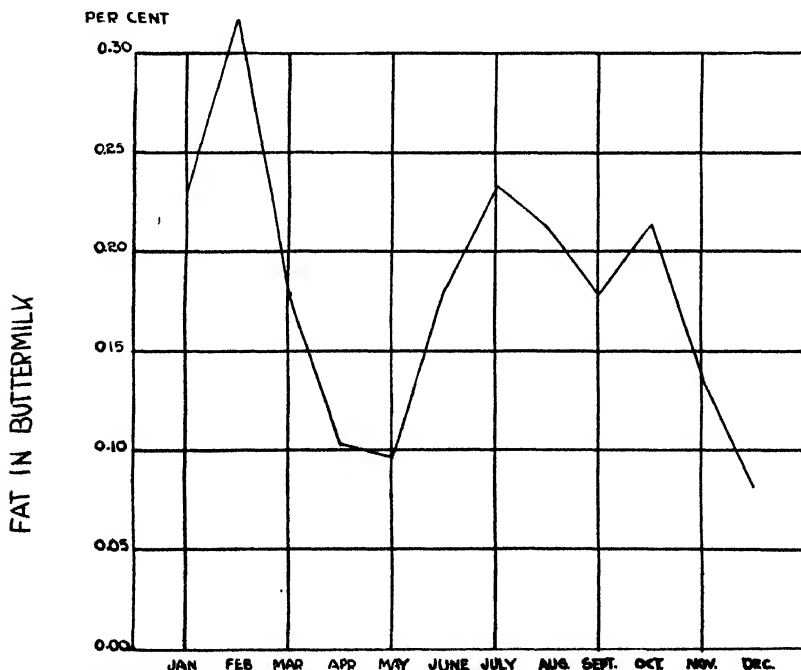


FIG. 1. MONTHLY AVERAGE FAT PERCENTAGES OF BUTTERMILK AT MINNESOTA STATE EXPERIMENTAL CREAMERY. 1927.

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* The data in this paper are taken in part from the thesis presented by S. T. Coulter in partial fulfillment of the requirements for the degree of Ph.D., University of Minnesota, 1933. Paper 1260, Journal Series, Minnesota Agricultural Experiment Station.

conditions, the fat content of the buttermilk was markedly reduced in the years that followed. This is shown by comparison of the data for 1927 (Fig. 1) with those for the years 1930 and 1931 (Figs. 2 and 3). The plotted values are the monthly average fat percentages of the buttermilk according to the Standard Babcock test. It is believed that the fat percentages during the years 1930 and 1931 represent, approximately, the minimum which it is possible to secure under commercial conditions when churning sweet cream untreated except for pasteurization.

There was a strikingly uniform seasonal change in the fat content of the buttermilk during the years from 1929 to 1932. The values for 1929 and 1932 are not shown on the charts but are similar to those from 1930 and 1931. The increase in the fat percentage during the months of May, June,

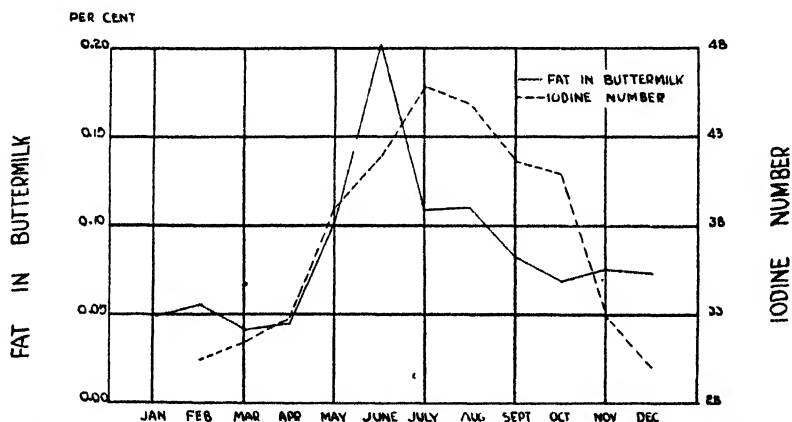


FIG. 2. MONTHLY AVERAGE FAT PERCENTAGES OF BUTTERMILK, AND IODINE NUMBERS OF THE BUTTERFAT FROM MONTHLY COMPOSITE SAMPLES OF BUTTER. MINNESOTA STATE EXPERIMENTAL CREAMERY. 1930.

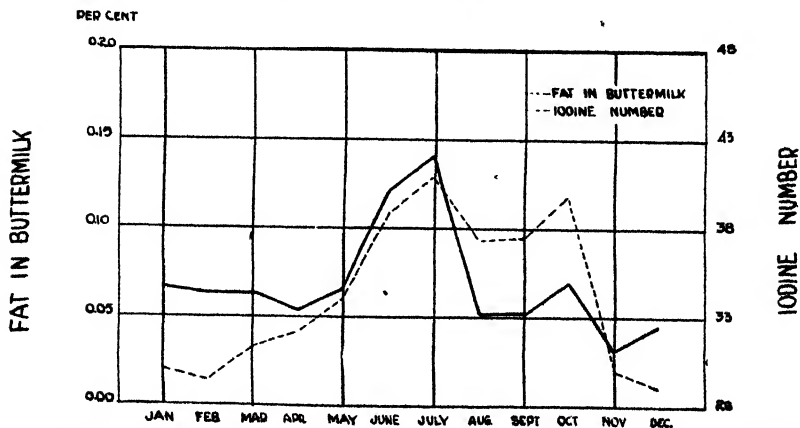


FIG. 3. MONTHLY AVERAGE FAT PERCENTAGES OF BUTTERMILK, AND IODINE NUMBERS OF THE BUTTERFAT FROM MONTHLY COMPOSITE SAMPLES OF BUTTER. MINNESOTA STATE EXPERIMENTAL CREAMERY. 1931.

and July persisted despite the use of extremely low churning temperatures with consequent prolonged churning periods. Buttermakers commonly use lower churning temperatures during this season because of the softness of the butterfat. The data indicate, however, that there is a seasonal variation in the maximum recoverability of butterfat from cream on churning. This variation appears to be associated with the seasonal changes in the composition of the butterfat. Monthly composite samples of the butter were secured during 1930 and 1931. The fat from the butter was rendered and the iodine number determined. The peak values for butterfat losses and iodine numbers were in June and July at which time the cows are fed almost exclusively on pasture. The rise in both values in October, 1931, is interesting. Heavy rains in this section during early October of that year, following a dry summer, revived the pastures. The resulting change in the character of the pasture grass or in the amount of grass consumed by the animals is reflected in the change in the composition of the butterfat.

These observations aroused interest in the relation between the composition of the butterfat and the churnability of the cream. Further studies were therefore made with the cream from the milk of individual cows.

Equipment, similar to that described by Guthrie and Sharp (1), was used for these studies on churnability. This consisted of a battery of four 2-gallon glass churns with contained agitators which were driven through gears from the same line shaft. The electric motor used to propel the line shaft was sufficiently powerful to permit the removal of one or more of the churns without changing the speed appreciably. The glass portion of the churns was almost entirely immersed in a water bath maintained at 11° C.

The combined milk produced by the individual cows during periods of two consecutive days was separated, standardized with skimmilk to 30 per cent butterfat, and held in the refrigerator at 5° C. for five hours. Six pounds of cream were weighed into the churns and held in the water bath at 11° C. for one hour before churning. The churning time was recorded and the percentage of fat in the buttermilk was determined by using the Minnesota reagent in the Babcock test. In order to avoid incorporating in the buttermilk any unchurned portions of the cream that may adhere to the top of the churn during the churning process, it was necessary to pipette or siphon sufficient buttermilk for analysis from the bottom of the churn.

Guthrie and Sharp (1) noted a difference in the time required to churn with different churns. This situation was not observed in the equipment used for this work. Excellent agreement, both as to churning time and the percentage of fat in the buttermilk, was consistently secured when churning different lots of the same cream.

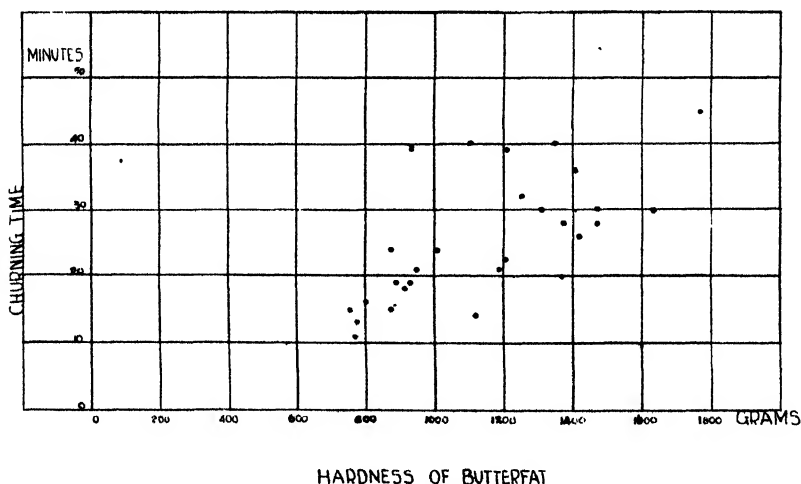
Determinations of the iodine and the Reichert-Meissl numbers were made on the rendered, filtered butterfat. In addition, the hardness of the butterfat was determined with the equipment described by Coulter and Hill (2).

Several investigators, including Coulter and Hill (2), have shown that the hardness of butter or butterfat is closely correlated with the iodine number of the butterfat. The hardness of butterfat increases with decrease in iodine number.

Two series of experiments were conducted, in the first of which, the hardness determinations were made at 11° C., and in the second at 12° C. In the first series, data were secured on 9 churnings from the cream of each of three cows, two Holstein and one Brown Swiss. In the second series, data were obtained on 42 churnings with cream from 5 Holstein, and from 2 Guernsey cows.

RELATION BETWEEN THE CHURNABILITY OF CREAM AND THE HARDNESS OF BUTTERFAT

The data secured in this work indicate that the time required to churn different lots of cream from the same cows is directly proportional to the hardness of the butterfat. This relationship is shown in figures 4 and 5 in which the churning times have been plotted as ordinates and the hardness of the butterfat samples as abscissas. The data in figure 4 are for series 1, and in figure 5 for series 2.



It was observed that the Guernsey cream, in series 2, churned in a shorter period of time than the Holstein cream containing butterfat of the same hardness. This is clearly indicated by the plotted data in figure 5. This result would seem logical since the fat in the milk of Guernsey cows

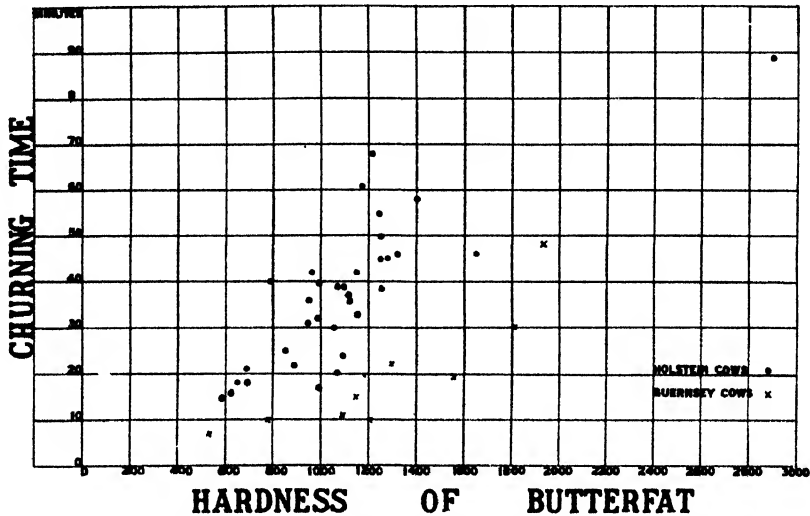


FIG. 5. RELATION BETWEEN THE CHURNING TIME OF CREAM AND THE HARDNESS OF BUTTERFAT. SERIES 2.

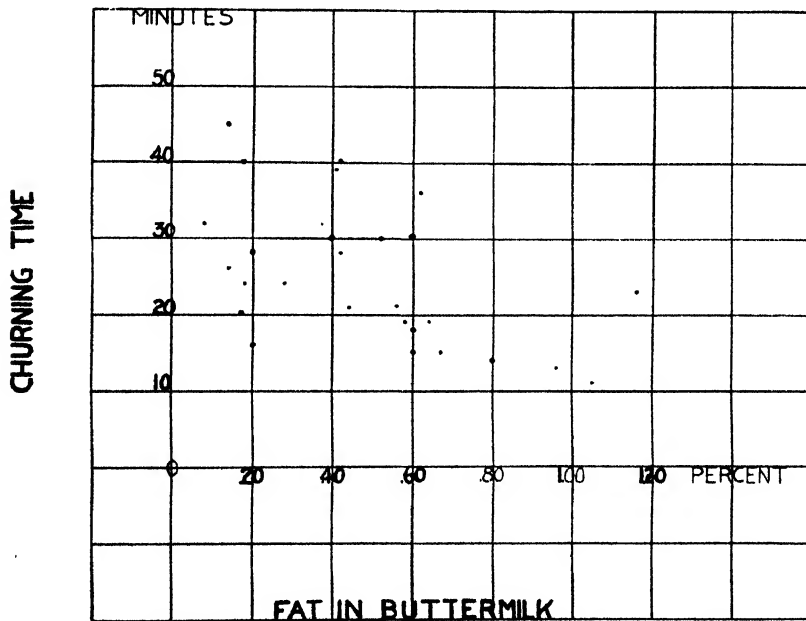


FIG. 6. RELATION BETWEEN THE CHURNING TIME OF CREAM AND THE FAT CONTENT OF THE BUTTERMILK. SERIES 1.

commonly occurs as larger globules than that from Holstein cows. Large fat globules churn more readily than small ones.

The relationship between the hardness of the butterfat and the churning time appears to be essentially linear except with the softer butterfats and the shorter churning time. Since cream containing butterfat with a hypothetical hardness of zero would not churn spontaneously but would require an interval of time for agitation, a regression line of churning time as determined by hardness of butterfat would start at some point greater than zero on the time axis. This is indicated by the plotted data.

The fat content of the buttermilk was the lowest in the samples requiring the longest time to churn. These samples contained the hardest butterfat. The data for series 1 are shown in figure 6, and for series 2, in figure 7.

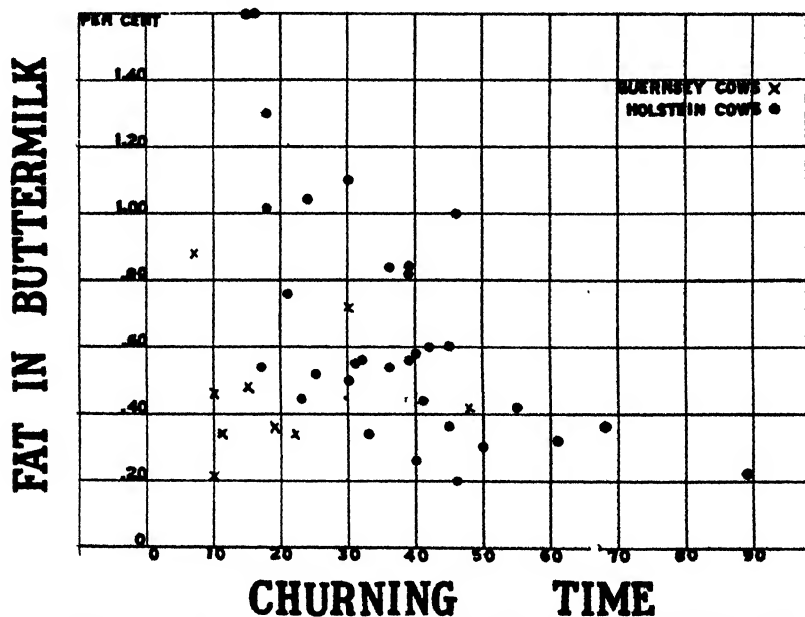


FIG. 7. RELATION BETWEEN THE CHURNING TIME OF CREAM AND THE FAT CONTENT OF THE BUTTERMILK. SERIES 2.

The fat percentages of the buttermilk samples are plotted as ordinates, and the churning times in minutes as abscissas. Although the results are quite variable, there is a definite tendency for the fat content of the buttermilk to increase with a decrease in churning time.

DISCUSSION

The data clearly show that the churnability of the cream is influenced by the hardness of the butterfat. Although hardness determinations were not made on butterfat from the monthly composite samples of butter

received from the State Experimental Creamery, the hardness of the butterfat may be expected to have followed closely the changes in the iodine number. Since the relation between the churning time of cream and the hardness of butterfat is essentially linear, alteration of the churning temperature may be expected to equalize the churning time with creams containing hard and soft butterfat. This is illustrated by the work at the State Experimental Creamery, where the churning temperature has been adjusted with the changing seasons to secure an almost constant churning time. In fact, during the years from 1929 to 1932 the churning time during the summer months has usually been longer than the churning time during the winter months. This is due to the very low churning temperatures used during the summer months. Although the churning time of cream containing hard and that containing soft butterfat may thus be equalized by alteration of the churning temperature, such adjustment to equalize the churning time does not appear to reduce the fat content of the buttermilk, when churning cream containing soft butterfat, to a point comparable with that for cream containing hard butterfat.

SUMMARY AND CONCLUSIONS

Data which indicate a seasonal variation in the fat content of buttermilk are presented. This variation appears to be associated with the seasonal fluctuations in the iodine number and thus with the changes in the hardness of the butterfat. It has been shown, experimentally, that the churning time of cream from the same cows is prolonged when there is an increase in the hardness of the butterfat, and also that the fat content of the buttermilk decreases with an increase in the time required for churning.

REFERENCES

- (1) GUTHRIE, E. S., AND SHARP, F. P. The effect of the hydrogen-ion concentration on the churning time of cream. *JOUR. DAIRY SCI.* 14: 1-13. 1931.
- (2) COULTER, S. T., AND HILL, O. J. The relation between the hardness of butter and butterfat and the iodine number of the butterfat. *JOUR. DAIRY SCI.* 17: 543-550. 1934.

THE OCCURRENCE OF POLYTHELIA IN DAIRY CATTLE*

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At the present period in the evolutionary development of dairy cattle, the females normally have four milk secreting glands, or mammae. Associated with each of these mammae is the papilla mamma, commonly called a teat or nipple, which serves as an exit for the secretion produced in the mammary gland. It is not uncommon, however, to observe individuals that have in addition to the four "normal" teats a variable number of additional teats, some of which are the orifices of small glands, others may be without glands and yet others may open into one of the normal glands. This condition of polythelia is usually referred to as supernumeraries, after teats, abortive teats, or extra teats.

There is a tendency for the majority of dairy cattle breeders in the United States to look upon polythelia with disfavor since the appearance of extra teats detracts from the beauty of the cow and also because the glands associated with the extra teats frequently produce small amounts of milk, thereby causing irregularities and difficulties in the milking process. A few breeders, however, believe that supernumeraries are associated with abundant milk and butterfat production.

Therefore, a genetic analysis of the inheritance of the supernumerary teats would be most valuable to dairy cattle breeders as an aid in the selection of their breeding animals so that they could decrease the frequency of the polythelial condition if it should be desirable. Before such a study is to be initiated, however, it would seem desirable to secure definite information as to the frequency of occurrence and the normal locations, which we may designate as pattern, of the supernumeraries as they now occur in our various breeds of dairy cattle.

Therefore, it was the plan of this investigation to collect data on large numbers of individuals in the various breeds of dairy cattle in order to secure the supernumerary teat patterns and the frequency of their occurrence. No attempt will be made in this paper to determine the genetic behavior of the factors causing the somatic appearance of these extra teats.

REVIEW OF LITERATURE

Without doubt supernumerary teats were observed by breeders at an early period in the development of dairy cattle, but a discussion of their

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occurrence, in the literature, seems to be limited to the recent three quarters century.

The first description that has been found by the author was given in 1862 by May (11). He mentioned the fact that there may appear one or two small pseudo teats on the rear glands that were considered favorable signs. Sutton (12) also described the polythelial conditions in the cow and other animals.

In a book on variation, Bateson (1) states that, "Normally the cow has four teats of about equal size. Not infrequently there are six teats of which four are large and may be said in the usual parlance to be the "normal" ones, and two are small and placed posteriorly to the others. . . . Their size and position vary greatly; sometimes they are placed near the other teats . . . but I have seen them very high up, almost in the fold between the udder and the thighs. Very frequently, however, there is only one extra teat, making five in all, such an extra teat being so far as I know, always on one side of the udder."

Burchard (2) compared the number of supernumeraries that occur in growing cattle with those found on the mammary gland of embryos. Approximately 38 per cent of all the individuals examined showed abortive teats, as he classified them.

Henneberg (6) made a very extensive study of the frequency of occurrence of supernumerary teats in various breeds of European cattle. He examined 2373 females and found that approximately 39 per cent of these animals possessed supernumeraries.

Mackenzie and Marshall (10) examined 276 dairy cows and heifers at the Cambridge University Farm and at seven other dairy farms for the occurrence of accessory or supernumerary teats. They found that the supernumeraries occurred in more than 50 per cent of the animals observed. The numbers varied from one to three.

Juler (8) reported 1472 observations on the breeds of dairy cattle in Germany and found that about 23 per cent showed the so-called "after teats."

Emmerson (3) examined the udders of 370 beef and dairy cattle and found that 44 per cent of the individuals exhibited supernumerary teats.

Ivanova (7) studied about 4000 cattle in U. S. S. R. for the occurrence and gave the various locations and patterns of supernumerary teat placements on 800 individuals, but did not indicate the percentage of animals that showed the characteristic.

Leroy (9) observed that among a group of 90 experimental cows representing the different breeds in France, there were only 28 cows that did not possess the supernumerary teats. In other words, approximately 69 per cent of the animals in this group exhibited the polythelial condition.

Turner (13 and 14) reported a study of 40 female and 46 male bovine

fetuses. Fifty per cent of the females had the supernumerary teats, while only 17 per cent of the males had more than the four rudimentaries.

A summary of the percentage of supernumerary teats of all kinds in relation to the normal number as reported by these various investigators is given in Table 1.

TABLE 1
Summary of the data on the occurrence of supernumerary teats in cattle

BREED	TOTAL NUMBER OF ANIMALS INCLUDED	TOTAL NUMBER OF ANIMALS WITH SUPERNU- MERARIES	PERCENTAGE OF ANIMALS WITH SUPERNU- MERARIES	AUTHORITY
Unclassified cattle . . .			37.62	Burchard (1897)
Embryos—male . . .			39.58	“ “
“ —female . . .			35.13	“ “
Holländer . . .	122	34	27.87	Henneberg (1904)
Ostfriesen . . .	339	94	27.73	“ “
Krenzung aus Holländer und Ostfriesen . . .	40	18	45.00	“ “
Oldenburger . . .	114	51	44.74	“ “
Shorthorn . . .	40	16	40.00	“ “
Rotbunte Schleswig-Hol- steiner . . .	132	57	43.18	“ “
Rote Schleswiger . . .	243	73	30.04	“ “
Braunveih . . .	215	84	39.07	“ “
Gelbe einfarbige Talland- rinder . . .	110	36	32.73	“ “
Einfarbig Rotes u. Rotbr. veih . . .	476	185	38.86	“ “
Braun- und Rotblassige Rinder . . .	18	4	22.22	“ “
Rückenschecken . . .	43	17	39.53	“ “
Grosse Hohenfleckveih . .	481	257	53.43	“ “
Dairy Shorthorns . . .	175	105	60.00	Mackenzie & Marshall (1925)
Devon . . .	81	40	49.38	“ “
Jersey . . .	8	5	62.50	“ “
Beef & Dairy Cattle . . .	370	163	44.05	Emmerson (1928)
Various breeds in Ger- many . . .	1472	338	22.96	Juler (1927)
Various breeds in France .	90	62	68.88	LeRoy (1928)
Embryos—female . . .	40	20	50.00	Turner (1930, 1931)
“ —male . . .	46	8	17.39	“ “ “
Various dairy breeds in Missouri— females . . .	4831	1249	25.85	(This paper)
males . . .	135	19	14.07	“ “

SOURCE OF DATA

This investigation was started by examining the number and distribution of the teats on all animals in the dairy herd at the Missouri Agricultural Experiment Station in 1926. These observations have been continued since that date at regular intervals. It was later found possible to obtain similar observations from more than 230 herds of dairy cattle in various sections of Missouri, through the cooperation of a carefully selected group of Advanced Registry supervisors and dairy herd improvement association

testers.¹ These men were given thorough instructions and were furnished with forms for facilitating the accuracy and ease of securing reports.

POSITION AND PATTERN OF SUPERNUMERARY TEATS

In general, there are three types of supernumerary teats that have been observed—caudal, or those found at the rear of the normal; intercalary or those found between the normal teats; and ramal, or the supernumeraries that are ramifications and branches of the normal teats. Supernumeraries anterior to the normal have not been observed.

In general, each of these three types have a rather definite maximum number of possible locations on the udder for supernumerary attachments. The caudal type may have four supernumeraries, one pair just posterior to the normal teats, and a second pair posterior to the first pair of supernumeraries and placed well up on the rear quarters of the udder, but may be near the median line. The intercalary supernumeraries are attached to the floor of the udder and may occur at various points between the normal teats on each half of the udder. They are usually in line with the two normal teats on each half of the udder. The ramal type appears at the base of the normal teat or on the side of the teat and may be attached on the inner or outer position, or caudal or cranial to the normal teat.

Figure 1 illustrates the three types of supernumeraries commonly ob-

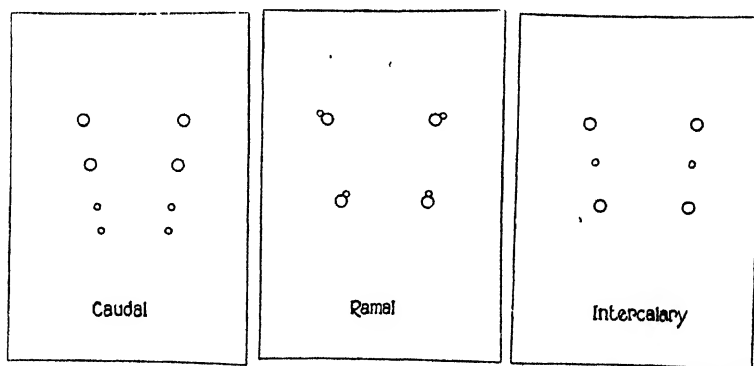


FIG. 1. DIAGRAMS ILLUSTRATING THE THREE TYPES OF SUPERNUMERARIES COMMONLY OBSERVED ON THE UDDERS OF DAIRY COWS

served and shows the most probable locations while figure 2 shows photograph of observations of each of these types.

One or more of the supernumeraries in each of the three types may occur on a single udder. Therefore, in order to facilitate the grouping and classi-

¹ This second procedure was initiated by Dr. Chas. W. Turner, Dairy Department, University of Missouri, and the writer is greatly indebted to him for about one-third of the observations obtained for this study and for other valuable suggestions.

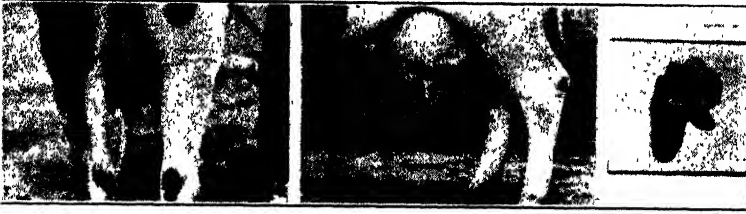


FIG. 2. PHOTOGRAPHS SHOWING THE VARIOUS TYPES OF SUPERNUMERARY TEATS

1. Two caudal supernumeraries
2. An intercalary supernumerary
3. An amputated teat showing ramal supernumerary.

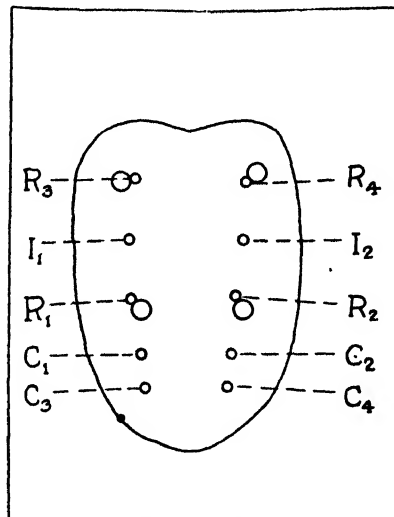


FIG. 3. DIAGRAM ILLUSTRATING THE HYPOTHETICAL POSSIBLE LOCATIONS OF SUPERNUMERARY TEATS ON A SINGLE UDDER WITH IDENTIFICATION NUMBERS

fication of supernumerary patterns on the various udders studied, figure 3 is presented giving the hypothetical possible locations on a single udder with identification numbers. It will be noted that those appearing on the left half of the udder are designated by odd numbers while those on the right are marked by even numbers.

The frequency of appearance of supernumerary teats in the caudal, intercalary, and ramal positions, as well as the various combinations of the three types that have been reported by various investigators, are summarized in table 2.

In the present investigation, 4831 female individuals have been observed. This number includes cows and heifers of the Holstein-Friesian, Jersey and Guernsey breeds and a number of animals of mixed or unknown breeding. A summary of the observations shows that 1249 of these animals

TABLE 2
Summary of the test patterns reported for various breeds of dairy cattle

BREED	0	CAUDAL				INTER-CALARY		INTER-CALARY AND CAUDAL		RAMAL	AUTHORITY
		1	2	3	4	1	2	1	2		
Holländer	88	16	16	1	0	0	2	0	0		Henneberg (1904)
Ostfriesen	245	41	45	4	1	2	1	2	0		"
Kreuzung aus Holländer und Ostfriesen	22	8	8	1	0	1	0	4	0		"
Oldenburger	63	23	23	1	1	3	0	2	0		"
Shorthorn	24	10	6	0	0	0	0	0	0		"
Rotbunte Schleswig-Holsteiner	75	34	20	1	0	2	0	3	0		"
Rote Schleswiger	171	40	31	1	0	0	0	1	0		"
Braunveih	132	54	29	0	0	0	0	0	1		"
Gelbe einfarbige Talland Rinder	74	22	14	0	0	0	0	0	0		"
Einfarbig Rotes u. Rotbr. Veih.	291	107	77	0	0	1	0	1	0		"
Braun-und Rotblassige Rinder	14	3	1	0	0	0	0	0	0		"
Ruckenscheuten	28	7	8	0	0	0	0	2	0		"
Grosses Hohenfleckveih	224	116	131	3	0	6	1	8	0		"
Dairy Shorthorn	70	52	48	5							Mackenzie & Marshall (1925)
Devon	41	15	23	2							"
Jersey	3	2	1	2							"
Beef & Dairy Cattle	207	83	71	9	0	8	0				Emmerson (1928)
Various breeds in U.S.S. Russia		371	377	22	0	3	0	0	0	27	Ivanova (1928)
Various breeds—embryos											
female	20	10	10	0	0	0	0	0	0	0	Turner (1930-31)
male	39	6	1	0	0	1	0	0	0	0	
Dairy breeds in Mo.											
female	3582	737	431	15	3	49	8			33	(This paper)
male	116	9	10								"

had one or more supernumeraries. These were grouped according to the pattern combinations illustrated in fig. 3. For example, if there were a caudal supernumerary on the left half of the udder and an intercalary on the right half, the pattern was designated as C-1 + I-2.

The frequency distributions and the location of supernumeraries observed as indicated by the pattern are given in table 3. All three types, caudal, intercalary, and ramal supernumeraries, were observed. In all, 36 different patterns were found and no doubt further observations will reveal that other combinations and patterns appear.

The caudal teats were far more numerous than the intercalary or ramal types. Of the 1249 cows exhibiting supernumerary teats, 1186 or 94.96 per cent possessed one or more caudal teats. This was 24.55 per cent of all cows observed. There were 737 or 15.25 per cent of the cows with only one caudal, 431 or 8.92 per cent with two caudal, 15 or 0.31 per cent with three and only 3 or 0.06 per cent with four caudal supernumeraries.

The intercalary teats only appeared on 56 or 1.16 per cent of the cows. Of these, 49 cows had only one while the remaining 7 had two. The ramal type was found to be most rare. Only 33 cows or 0.68 per cent were observed with this type of abnormal teats. Furthermore 24 of these cows had only one while 8 had two and only 1 possessed three.

With the above distributions of types of supernumeraries, it is quite natural that patterns C-1, C-2 and C-1 + C-2 should be most frequent. It will be noted from the distribution table that 1084 of the cows were actually classified in these three patterns.

In the other 33 patterns, the numbers are small. In no case were there more than 0.4 per cent of the total animals observed in any of these individual patterns. In fact there are eleven of these patterns with only one animal represented.

One unusual pattern, C-1 + C-2 + C-3 + C-1¹, was observed where a caudal supernumerary was attached to the rear quarter to the left of the normal location of caudal supernumeraries.

Another cow was observed that had only three normal teats. The teat on the left half of the udder served as an exit for the milk secreted in the entire half of the mammary gland. Emmerson (3) reported similar observations and Heizer (5) reports a number of such individuals that have appeared in a Guernsey family.

Emmerson (3) also reports the more unusual type of udder. Only two teats were present to drain the four glands.

Erizan (4) reports three observations where cows had only two teats. The first one described had two normally developed teats and the arrangement indicated that they formed the anterior pair. Both teats functioned normally and there was no indication of rear or supernumerary teats located on the posterior portion of the udder. This udder is shown in

TABLE 3
The occurrence and patterns of supernumeraries observed in dairy cows

TEAT PATTERN	PUREBRED JERSEY	GRADE JERSEY	PUREBRED HOLSTEIN-FRIESIAN	GRADE HOLSTEIN-FRIESIAN	PUREBRED GUERNSEY	MIXED BREEDS AND MISCELLANEOUS	TOTAL
Normal (4 only)	541	1290	321	1153	52	225	3582
C-1	21	95	34	198	9	29	386
C-2	21	93	30	114	11	48	317
C-1+C-2	33	96	63	135	7	47	381
C-3	2	1	1	9	0	0	13
C-4	3	1	1	2	0	1	9
C-3+C-4	2	2	1	7	0	3	18
C-1+C-3	1	5	1	2	0	0	4
C-2+C-4	0	1	0	0	0	0	1
C-1+C-3+C-2	0	3	2	1	0	0	6
C-2+C-4+C-1	0	3	1	1	0	1	6
C-3+C-2+C-4	0	0	0	0	0	1	1
C-1+C-3+C-4	0	0	0	2	0	0	2
C-1+C-3+C-2+C-4	0	0	0	4	0	1	8
C-3+C-2	0	2	1	2	0	0	4
C-1+C-4	1	0	1	0	0	1	3
I-1+I-2	0	0	6	9	0	1	17
I-1	1	0	1	9	0	1	14
I-2	1	2	1	2	1	0	8
I-1+C-1	0	0	0	0	0	0	0
I-2+C-2	0	0	0	0	0	0	0
I-1+I-2+C-1+C-2	0	2	0	0	0	1	3
I-1+I-2+C-3+C-2	0	0	0	0	0	0	0
I-1+C-2	0	0	1	0	0	0	1
I-1+C-1+C-2	0	2	0	0	1	0	3
I-2+C-1	0	0	0	0	0	0	0
I-2+C-1+C-2	0	2	2	4	0	0	9
R-2	1	3	0	3	1	1	10
R-1+R-2	1	3	0	2	1	3	8
R-1	1	4	0	2	0	0	7
R-3	0	0	0	0	0	0	0
R-4	1	0	1	0	0	0	2
R-1+R-2+R-3	0	0	1	0	0	0	1
R-3+C-1	0	1	0	0	0	0	1
R-1+C-3	0	1	0	1	0	0	2
R-2+C-1	1	0	0	0	0	0	1
C-1+C-2+C-3+C-1 ¹	0	0	0	0	1	0	1
3 normal	0	1	0	0	0	0	1

figure 4. In the case of the second and third observations, the two teats occupied a central position on the udder and represented an incomplete blending of the rear and front teats into one system similar to the observation made by Emmerson.

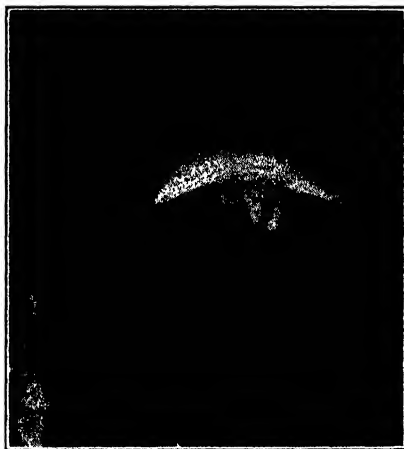


FIG. 4. PHOTOGRAPH OF A TWO-TEATED COW (FROM ERIZIAN)

THE OCCURRENCE OF POLYTHELIA IN BULLS

The supernumerary teats can also be observed on a number of dairy sires. The patterns in relation to the normal rudimentary teats are similar to those of the female patterns except these extra teats are usually located on the scrotum.

The small number of available bulls did not permit an extensive study of the supernumerary teat patterns of dairy sires. However, 135 bulls were examined. Of this number, 19 individuals or 14.07 per cent possessed

TABLE 4
Teat patterns observed in dairy bulls

TEAT PATTERN	PUREBRED JERSEYS	PUREBRED HOLSTEIN- FRIESIAN	PUREBRED GUERNSEY	MIXED BREEDS AND MISCEL- LANEOUS	TOTAL
Normal (4 only)...	60	40	2	13	115
C-1	1	2	0	1	4
C-2	4	1	0	0	5
C-1-C-2	3	4	2	1	10
3 Rudimentaries (only)	0	1	0	0	1

one or more supernumeraries. The various patterns observed, when classified according to the patterns illustrated in figure 3, are given in table 4.

Only three patterns were observed and these were of the caudal type. One young Holstein-Friesian bull was observed that had only three normal rudimentaries. A similar observation was made by Turner (14) as he reports a male fetus from a Hereford dam that had only three rudimentaries.

SUMMARY

The frequency of occurrence and pattern distribution of supernumerary teat observed on 4831 female and 135 male dairy cattle have been compiled and reported. For the females, 25.8 per cent exhibited the polythelial condition. The caudal, intercalary and ramal supernumeraries were observed and were classified in 36 different patterns. The caudal type was by far the most common type observed since approximately 95 per cent of all females possessing the extra teats were of this type. Only 14.07 per cent of the males had one or more supernumeraries and these were classified in three patterns. One individual was observed with only three rudimentaries.

Data of similar investigations made by European workers and others have been compiled. On the average the frequency of occurrence of the polythelial condition found in the American breeds studied were less than that reported for the European breeds.

REFERENCES

- (1) BATESON, WM. Materials for the study of variations. London. 1894.
- (2) BURCHARD, G. Über embryonale Hypermastie und Hyperthelie. Anat. Hefte., 8: 527-555. 1897.
- (3) EMMERSON, M. A. Studies on the macroscopic anatomy of the bovine udder and teat. Thesis, Iowa State College. 1928.
- (4) ERIZIAN, C. Das Vorkommen einiger Neubildungen bei Bos. taurus in S. S. R. Armonia. Zeitschr. f. Zuchtung. B. 22: 114-121. 1931.
- (5) HEIZER, E. E. An inherited udder abnormality in cattle. Jour. of Heredity, 23: 111-114. 1932.
- (6) HENNEBERG, B. Zur Kenntnis der Abortivzitzen des Rindes. Anat. Hefte, 25: 680-699. 1904.
- (7) IVANOVA, O. A. Über Vererbung der Mehrzitzigkeit beim Rind. Zeitschr. f. Tierzuch. u. Zuchtungsbiol. 11: 119-139. 1928.
- (8) JULER, J. Beitrag zur Kenntnis der afterzitzen des Rindes, ihre Bewertung als Milchzeichen und ihr Verhalten im Erbganze. Zeitschr. f. Tierzuch. u. Zuchtungsbiol. 10: 377-417. 1927.
- (9) LEROY, M. ANDRE. Contribution l'étude de la valeur pratique des signes laiters et beurriers chez la vache. Le Lait 9: 350-358. 1928.
- (10) MACKENZIE, K. J. J., AND MARSHALL, F. H. A. On the presence of supernumerary

mammary glands in cows and on their functional activity. Jour. Agr. Sci. 15: 30-35. 1925.

- (11) MAY, G. Das Rind, (München) 1: 428. 1862.
- (12) SUTTON, J. B. Supernumerary mammae and nipples in man, monkeys, cows, etc. Am. Jour. Med. Sci. 97: 247-257. 1889.
- (13) TURNER, C. W. The anatomy of the mammary gland of cattle. Embryonic development. Mo. Agr. Exp. Sta. Res. Bul. 140. 1930.
- (14) ————. The anatomy of the mammary gland of cattle. Fetal development. Mo. Agr. Exp. Sta. Res. Bul. 160. 1931.

THE BUTTERFAT RECORDS OF COWS POSSESSING SUPERNUMERARIES COMPARED WITH COWS HAVING THE NORMAL NUMBER OF TEATS*

WARREN GIFFORD

Missouri Agricultural Experiment Station, Columbia, Missouri

It is not an uncommon occurrence to have dairy cattle breeders question whether or not cows with supernumerary teats are superior in producing capacity to those that do not show the polythelial condition. As was described in an earlier paper, Gifford (2) these "extra teats" or supernumeraries are found quite commonly in dairy cattle and are usually of three types. They have been described as caudal, or those found in the rear of the normal teats, intercalary or those found between the normal, and ramal or those that are ramifications or branches of the normal.

In Russia and some other European countries, it has been reported that there is a prevailing belief among dairy cattle breeders that the presence of supernumeraries is evidence of abundant milk supply. In other words, the observations of these breeders have led them to believe that there is closely linked with the genes for the character capacity for large amounts of milk, the genes responsible for the development of the polythelial condition. Furthermore, their conclusion might indicate that the genes responsible for the development of these two characters are so closely linked that they appear together in a majority of instances. In fact, Ivanova (3) found that the cows possessing supernumerary teats had produced 388 kilograms or about 15 per cent more milk than those with the normal number of teats when she compared the records of 251 cows that exhibited this polythelial condition with 444 cows with the normal number. These cows were observed in 25 dairy herds on farms and at breeding stations in U. S. S. Russia.

On the other hand, Juler (4) working independently of Ivanova made a similar investigation on the records of the Angler cattle in Germany and the results were quite contradictory. Juler failed to find a significant difference between the annual production of milk and butterfat of the two groups. Leroy (5) substantiated Juler's findings when he examined 90 cows for the presence or absence of supernumerary teats and then compared the milk production of the two groups, taking as a basis for his comparison, the average mature or mature equivalent records for three successive lactations. The group of cows with the normal number of teats actually produced considerably more milk on the average than the other groups. The author

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suggests, however, that there is not a significant difference between the two groups.

During the compilation of the data used to determine the frequency of the occurrence of the polythelial condition in breeds of dairy cattle represented in Missouri, it was possible to secure the annual butterfat records for seven groups of dairy cows. Each group represented a breed or a subdivision of a breed upon which record had been kept in a definite organized manner. The total number observed with such records was 1081 cows.

The records of these cows were corrected to a common age basis by means of the conversion factors published in the Missouri Agricultural Experiment Station Bulletin No. 274. Then for each of the groups, the records were divided into two subgroups, those that were made by cows with one or more supernumerary teats and those made by cows with the normal number of teats. The mean of the records in each of these subgroups and the standard deviations were determined. These data are presented in

TABLE 1
A Comparison between the annual fat production of dairy cows with only four teats and those with supernumeraries

GROUP OF COWS	BREED	FOUR NORMAL TEATS SUPERNUMERARIES ABSENT			FOUR NORMAL TEATS ONE OR MORE SUPER- NUMERARIES			DIFFERENCE IN POUNDS
		Num- ber	Mean fat produc- tion	Stand- ard devia- tion	Num- ber	Mean fat produc- tion	Stand- ard devia- tion	
1	Grade Jerseys	237	304.72	92.76	70	297.97	91.12	- 6.75
2	Purebred Jerseys	36	258.25	85.48	6	251.81	40.97	- 6.44
3	Grade Holsteins	264	299.31	85.07	129	296.59	71.01	- 2.72
4	Purebred Holsteins	48	320.18	103.59	25	305.15	83.28	- 15.03
5	Miscellaneous	45	258.53	78.82	35	265.97	76.77	7.44
6	R. of M. Jerseys	67	538.08	104.82	8	657.59	92.63	119.51
7	A.R.S.O. Holsteins	84	613.83	131.74	27	630.35	124.33	16.52

table 1. The average difference in pounds of fat between the two subgroups of records being compared are also shown in this table.

It will be noted from this table that in groups 1, 2, 3, and 4, respectively, the cows with the normal number of teats have a slightly superior yearly production on the average than do the cows with supernumeraries. In groups 5, 6, and 7, the situation is reversed and the cows with the supernumeraries have the highest average production. In order to determine whether these differences were significant or whether they may have arisen from mere fluctuations in sampling, *t* values were calculated and the sig-

nificance determined by Fisher's tables (1). That is the $\sigma MD = \sqrt{\frac{\sigma_1^2}{N_1} + \frac{\sigma_2^2}{N_2}}$ when σ_1 and σ_2 represents the standard deviation of subgroups 1 and 2 respectively and N_1 and N_2 represent the number of records in each of the

two subgroups. Then $t = \frac{\text{Difference}}{\sigma M.D.}$. These constants are given in table

2. It will be noted from the t values that the differences observed between

TABLE 2
Constants showing the significance of differences found between the two groups of records

GROUP	DIFFERENCE IN POUNDS BETWEEN THE FAT PRODUCTION OF THE TWO GROUPS	DEGREES OF FREEDOM	VALUE OF T	SIGNIFICANT
1	6.75	305	.5423	No
2	6.40	44	.2931	No
3	2.72	391	.3335	No
4	15.03	71	.6715	No
5	7.44	78	.4250	No
6	119.51	73	3.3986	Yes
7	16.52	109	.5918	No

the groups of cows exhibiting the supernumerary trait and those with the normal number of teats are not significant except in the case of group 6. Although the difference found between the means of production of these subgroups of this particular sample of purebred Jersey Register of Merit cows is highly significant, there is some doubt as to whether the sample is representative of a larger sample of cows in the same breed. Since there are only eight cows with the supernumerary teats in this group and since group 2 is made up of cows of the same breed and does not show similar results such a doubt seems most logical.

When all the observations are considered, it seems that the factors for the capacity for high butterfat production are not closely linked with the character supernumerary teats. From the practical viewpoint, there is no indication that supernumeraries are external traits indicating ability superior to the normal condition for butterfat production.

LITERATURE CITED

- (1) FISHER, R. A. Statistical methods for research workers (Fourth edition). Oliver and Boyd. 1932.
- (2) GIFFORD, W. The occurrence of polythelia in dairy cattle.
- (3) IVANOVA, O. A. Über Vererbung der Mehrzitzigkeit beim Rind. Zeitschr. f. Tierzuch u. Zuchtungsbiol. 11: 119-139. 1928.
- (4) JULEB, J. Beitrag zur Kenntnis der Afterzitzen des Rindes, ihre Bewertung als Milchzeichen und ihr Verhalten im Erbgauze. Zeitschr. f. Tierzuch. u. Zuchtungsbiol. 10: 377-417. 1927.
- (5) LEROY, M. A. Contribution à l'étude de la valeur pratique des signes lacters et beurriers chez la vache. Le Lait 9: 350-358. 1928.

PROCEEDINGS OF THE 29TH ANNUAL MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

R. R. GRAVES

Secretary

The 29th Annual Meeting of the American Dairy Science Association was held on the campus of Cornell University, at Ithaca, New York, on June 26th and 27th, and on the 28th the convention moved to the New York Agricultural Experiment Station, at Geneva, New York, for the third and last day of the conference.

At the 28th Annual Meeting at the University of Illinois last year, we recalled and discussed the organization meeting of our association at that place in the summer of 1906, and the part played by Professor W. J. Fraser in calling this meeting. The second annual meeting of what was then the "National Association of Dairy Instructors and Investigators" was called to order by President R. A. Pearson in the Record Building at Chicago, Illinois, on October 11, 1907. Eleven were present when the meeting was called to order and 21 were present at the banquet that evening.

Reports from the following committees were received and discussed :

1. Dairy score card.
2. Our relations to the National Dairy Show.
3. Courses of instruction.
4. Experimental work : Production, Manufactures.
5. Membership.

The officers elected at the meeting were :

President, R. A. Pearson (reelected).

Vice-President, Oscar Erf.

Secretary-Treasurer, C. B. Lane.

In the course of his address, President Pearson remarked :

The name of our organization is not descriptive of its character. We call it "The National Association of Dairy Instructors and Investigators," yet some of our members represent the great dairy interests of Canada. It is then, strictly speaking, not a National Organization. The name may be criticised also, because it is cumbersome. I would like to suggest as a new name "Official Dairy Instructors' Association." . . .

. . . . We do not want to have a poorly attended meeting annually if we can have a well attended meeting every two years. Until our work requires annual meetings I respectfully advise a meeting for business and the election of officers every two years. . . .

The Proceedings of the Third Annual Conference at Cornell University, Ithaca, New York, July 22, 1908, were as follows :

Remarks by President.

Reports of Committees :

1. Dairy Score Card.
2. Official Testing of Dairy Cows.
3. Our Relations to the National Dairy Show.
4. Courses of Instruction.
5. Experimental Work in Milk Production.
6. Experimental Work in Dairy Manufactures.
7. Standards for Dairy Products.
8. Official Methods of Testing Dairy Products.
9. Relations to Dairy Supply Houses.
10. Co-operative Work.
11. Extension Work.
12. Judging Dairy Cattle.
13. Cow test Associations.

A photograph was taken of those in attendance at this Third Annual Meeting and there were 47 men appearing in the picture. Compare this attendance with that of the 29th Annual Meeting at Cornell University where there were approximately 250 men registered. There were more than a hundred women and children came with Dad to the meeting.

At the Banquet on Wednesday evening President Stoltz asked those who were present who attended the Third Annual Meeting at Cornell University to stand. They were :

1. P. A. Campbell, then at Orono, Maine, now at Springfield, Mass.
2. H. E. Ross, then and now at Cornell University.
3. C. C. Hayden, then at Urbana, Illinois, now at Wooster, Ohio.
4. A. B. Nystrom, then at Manhattan, Kansas, now at Washington, D. C.
5. E. S. Guthrie, then and now at Cornell University.
6. J. H. Frandsen, then at Moscow, Idaho, now at Amherst, Mass.
7. O. F. Hunziker, then at Lafayette, Indiana, now at Chicago, Illinois.
8. H. C. Troy, then and now at Cornell University.

There were three others who attended the 1908 meeting who attended this year's meeting. They were :

1. H. H. Wing, then and now at Cornell University.
2. C. W. Larson, then at State College, Pa., now at Buffalo, N. Y.
3. E. S. Savage, then and now at Cornell University. (Professor Savage was in a hospital in Ithaca, with a serious illness during this year's meeting.)

At the business meeting of the association on the morning of the 26th, Dean Carl E. Ladd, of Cornell University, was introduced by Doctor Sherman. Dr. Ladd welcomed the members of the association to the campus

at Cornell University and gave an interesting discussion of the investigational work under way that bears upon the dairy industry; of the men who had been associated with Cornell University who had made history in the industry; something of the student body and other interesting phases concerning the work of the University.

R. R. Graves, Secretary-Treasurer of the association, made a brief report in which he stated that the present membership of approximately 600 members was the highest in the history of the association and was some 65 per cent higher than at the same date a year ago.

Since the minutes of the last meeting at Urbana, Illinois, were presented in full in the November Issue of the 1933 JOURNAL OF DAIRY SCIENCE the reading of the minutes were omitted.

President Stoltz appointed the following Pasture Committee to work on pasture problems:

Dr. G. Bohstedt	Wisconsin.
Dr. I. R. Jones	Oregon.
C. B. Bender	New Jersey.
R. B. Becker	Florida.
J. W. Linn	Kansas.
R. H. Lush	Louisiana.

This committee was appointed by the President of the association due to the fact that its work was of interest not only to the members of the Production Section but also to those of the Extension Section. It was explained that one representative of the American Dairy Science Association was to be appointed to work on a joint committee with members representing the Animal Production Society and the Agronomic Society. R. H. Lush, of Louisiana, was appointed to serve on this joint committee as the representative of the American Dairy Science Association.

President Stoltz announced that the annual meeting for 1935 would be held at the University of Minnesota, St. Paul, Minnesota.

Doctor Jackson, Chairman of the Nominating Committee, consisting of Ellénberger, of Vermont; Nelson, of Montana; LaMaster, of South Carolina, and Nevens, of Illinois, appointed by the President earlier in the year, gave the report of the committee.

The nominations for officers to be elected by mail votes to be sent out to the members in August, as reported by the Nominating Committee were as follows:

For Vice-President:

H. A. Ruehe, Univ. of Illinois.

E. S. Guthrie, Cornell University.

For Director for three-year term:

R. B. Becker, Univ. of Florida.

M. Mortensen, Iowa State College.

The new By-laws provide that the Vice-President of the Association shall automatically become President for 1 year following the completion of his term of office as Vice-President. Dr. C. L. Roadhouse, the present Vice-President, therefore, succeeds Professor R. B. Stoltz as President, on October 1st.

The association approved the action of the Board of Directors taken at the meeting the previous evening on the following items: (1) reappointment of Journal Management Committee for the ensuing year, 1935, consisting of Graves, Borland and Hunziker, and (2) approval of the recommendation contained in the annual report of the Sec.-Treas. to the effect that the fiscal year of the American Dairy Science Association be changed to the calendar year, balancing the books of the association as of December 31 instead of November 30 as in the past.

Doctor Dahlberg, Editor of the JOURNAL, made a brief report concerning the affairs of the JOURNAL.

President Stoltz appointed a Resolutions Committee consisting of Dr. E. C. Thompson, *Chairman*, O. E. Reed, Roger Morse, A. C. Ragsdale and W. J. Fraser.

The general program, as printed in the June issue of the JOURNAL OF DAIRY SCIENCE, then got under way,

Following the afternoon program, Professor Harrison showed groups of animals of the different breeds represented in the University herd and discussed their breeding and the plans for the future development of the herd.

The program committee had planned a recreation picnic supper at which the members and their families were to be the guests of the Dairy and Animal Husbandry Departments, for the afternoon of the first day at Taughannock Falls Park. A rather steady rain, however, made it necessary for the committee to change the scene of this picnic supper to the livestock pavilion at the University. This picnic was greatly enjoyed by the members of the association and though the scenery was missing that might have made Taughannock Falls Park very interesting, the pavilion offered very good facilities for visiting among the members. One of the events of the evening was the presentation of a scroll to Dr. O. F. Hunziker, showing the appreciation of the association of his achievements, and of his contributions to the dairy industry and to the association. Doctor Dahlberg made the presentation speech and Doctor Hunziker responded with a most interesting talk in which he recalled his student days at Cornell University. The scroll presented to Doctor Hunziker contained the following wording:

HONORED

by several national governments for his outstanding leadership in dairy research and education; recognized by his co-workers as one of the ten master minds of dairying; kindly, lovable, inspirational, ever helpful and active in our Association

OTTO FREDERICK HUNZIKER

is given this tribute at the annual meeting at Ithaca, New York, on the twenty-seventh day of June in the year one thousand nine hundred and thirty-four by the

AMERICAN DAIRY SCIENCE ASSOCIATION

R. R. GRAVES, *Secretary*

R. B. STOLTZ, *President*

At the business meeting on June 27 the following report of the resolution committee was adopted:

Be it resolved that the American Dairy Science Association express their appreciation of the valuable services rendered this Association by the late Mr. K. W. Schantz, of K. W. Schantz, Inc., and convey to the members of his family our deep and sincere sympathy.

Be it resolved that the American Dairy Science Association express their appreciation of the valuable services rendered the dairy industry by the late Professor E. H. Farrington. Mindful of his many years of continuous service in systematizing scientific literature so that it could be readily understood, in translating with unusual ability the scientific results of research into plant practice, and in stimulating students of science to enter the then new field of dairying, we recognize him as one of the outstanding men of his time and extend to his wife and daughter our deep and sincere sympathy.

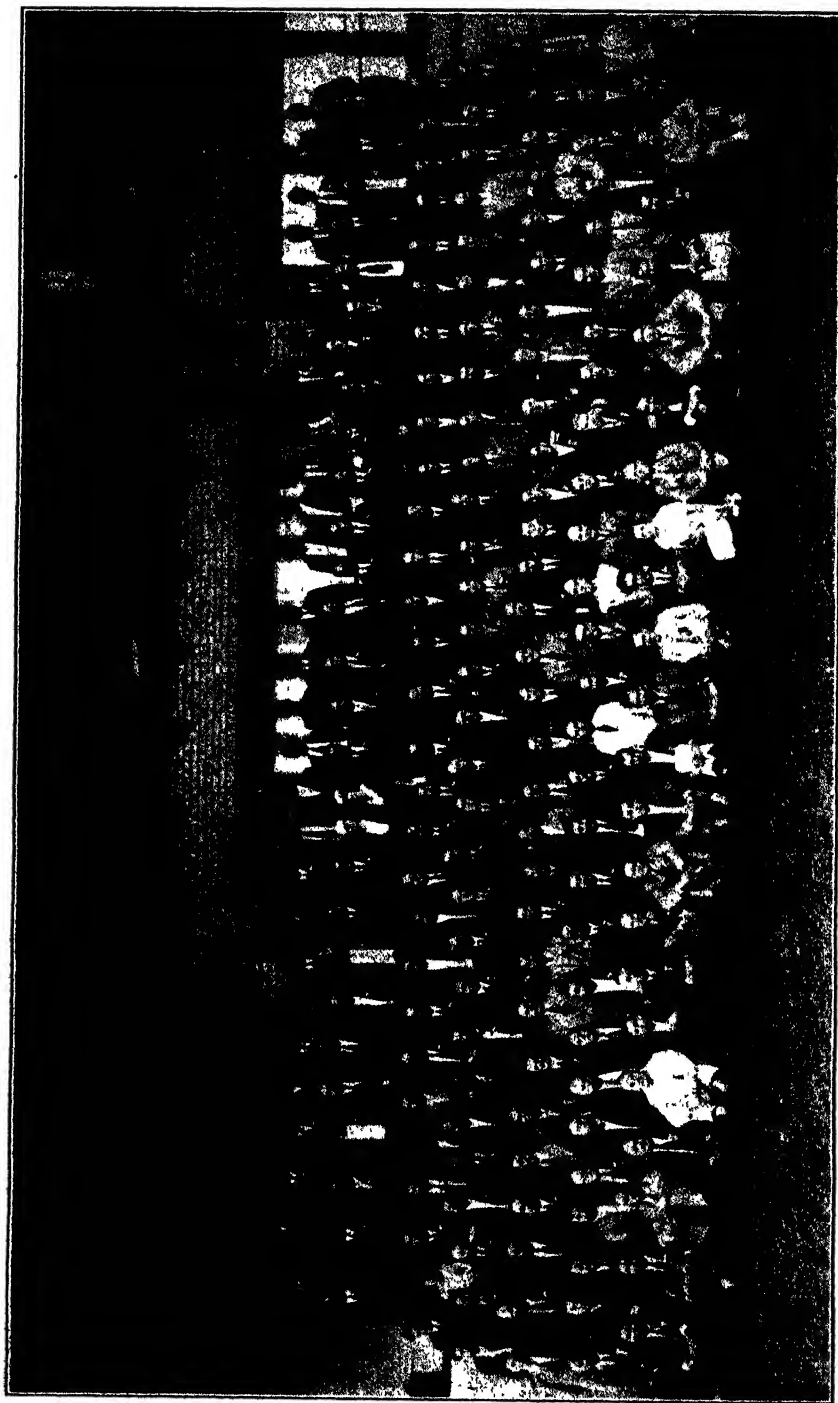
Be it resolved that the American Dairy Science Association express to Doctor E. S. Savage their sincere regret upon his inability to be with us upon this occasion and the sincere hope of a speedy recovery.

Whereas the per capita consumption of butter in this country is far below that of the leading butter producing countries of the world and this under consumption constitutes a serious problem to our industry, the permanent solution of which makes imperative the production of butter on the basis of quality, therefore: be it resolved that the American Dairy Science Association is opposed to any system or practice of production and procurement of cream and of the manufacture and distribution of butter that is known to jeopardize the quality of the finished product and we pledge our united support of and hearty cooperation with all sincere and fundamentally sound efforts on the part of the industry and regulatory authorities to combat trade practices detrimental to quality and we heartily approve the efforts of the Federal Food and Drug Administration involving the systematic and rigid inspection of all butter for purity and wholesomeness, and it is the sense of this association that the constructive and permanent improvement of the quality of cream can be made possible only by the purchase of all cream on the basis of quality.

Be it resolved that the American Dairy Science Association recognizes the importance of a great emergency and a long time agricultural adjustment program with special reference to the dairy industry and

We commend the efforts of the National Administration to develop and carry out such a program and especially the Dairy Section of the A. A. A., and the Dairy Bureau of the U. S. D. A. for their readiness to consult with and consider the viewpoint and interests of the dairy industry as a whole and,

We commend and would suggest enlargement of their educational activities relating



MEMBERS ATTENDING THE MEETING IN ITHACA ON JUNE 27, 1934

to the food value of milk and its products and in this connection their cooperation with the National Dairy Council and,

We approve and support a program for the control of diseases of dairy cattle and urge that to make it most effective and permanent an educational program to be projected either in advance of or at least concurrently with such a program to acquaint dairymen with the nature and control of the diseases included in the program.

In the case of Bang's disease, we suggest that indemnities be paid only to dairymen who sign an agreement with their State authorities to continue testing and control measures under their State accreditation rules for at least three consecutive years, and

We suggest that the secretary be authorized to send a copy of this resolution to Henry Wallace, Secretary of Agriculture, Chester Davis, of the A. A. A., and A. H. Lauterbach, chairman of the Dairy Section of the A. A. A.

Be it resolved that the American Dairy Science Association express their sincere appreciation to the College of Agriculture of Cornell University, to the Agricultural Experiment Station, to the Departments of Dairying and Animal Husbandry, to the Program Committee and to the Ladies of the Faculty as well as other individuals who have cooperated in furnishing the splendid provisions for the comfort and entertainment of all upon the occasion of the 29th Annual Meeting of the Association.

Be it resolved that the American Dairy Science Association with much appreciation commend the efforts of the Journal Management Committee and the editor of the JOURNAL OF DAIRY SCIENCE that have resulted in placing the JOURNAL on a monthly basis.

Be it resolved that the Association with much pleasure recognize the continued confidence and support given by the several advertisers in the JOURNAL OF DAIRY SCIENCE. We recognize that the JOURNAL is one of our major contributions to Dairy Science and to the Dairy Industry and on behalf of the industry we desire to express our appreciation for their loyalty and we trust that this JOURNAL will continue to merit their support.

Respectfully submitted,

O. E. REED
ROGER MORSE
A. C. RAGSDALE
W. J. FRASER
E. C. THOMPSON.

The following report of the Production Section was adopted:

The Production Section sessions have been conducted in accordance with the outlined program with every paper being given.

The business session for the section was held at the close of this morning's program. Reports were heard from the Breed Relation Committee which made recommendations concerning certain needed changes in the Herd Improvement Tests and from the Committee on Students Judging Contests. This latter committee recommended that consideration be given to the recommencing of the National Students Judging Contest in the year 1935.

The section then proceeded to the election of officers for 1935 with C. Y. Cannon, Iowa, being elected as chairman and R. H. Lush of Louisiana as secretary.

H. O. HENDERSON, *Chairman*.

The following report of the Extension Section, presented by James Linn, was adopted:

The Extension Division had a very full program, practically all of the papers being presented. A new departure, put into effect this year for the first time, was very successful. This was termed an exhibit of ideas and consisted of exhibits showing the extension activities of various States. These exhibits showed the material used in the

presentation of campaigns for increasing the consumption of dairy products, the organization of bull associations, cow testing associations, breeding schools, breeding demonstrations, etc.

The new officers elected by the Extension Service for the following year are Floyd Johnston, Iowa State College, Ames, Chairman, E. J. Perry, Jersey Experiment Station, Vice-Chairman, C. L. Blackman, Ohio State University, Secretary.

Owing to the fact that the Manufacturing Section did not have its business meeting till the last day at Geneva its report was not presented at the meeting but is presented herewith.

Dr. James Sherman, Cornell University, presented the following motion which was adopted after some discussion: "That a permanent committee be appointed to pass on honors conferred on individuals at annual meetings; that this be a standing committee consisting of the last three presidents and that the committee make its report to the Board of Directors."

President Stoltz discussed the membership drive in which he had taken an active part during the past year and presented a table showing the distribution of the membership of the association by States. The chart that was presented by President Stoltz follows:

	1933	1934		1933	1934
Alabama	1	1	New York	45	70
Arizona	1	7	North Carolina	1	4
Arkansas	1	1	North Dakota	3	3
California	25	36	Ohio	23	49
Colorado	2	0	Oklahoma	3	4
Connecticut	6	17	Oregon	5	9
Delaware	0	0	Pennsylvania	17	39
Florida	5	5	Rhode Island	0	0
Georgia	2	3	South Carolina	2	9
Idaho	3	2	South Dakota	1	2
Illinois	31	45	Tennessee	3	8
Indiana	15	20	Texas	4	4
Iowa	19	18	Utah	2	3
Kansas	8	10	Vermont	8	17
Kentucky	6	3	Virginia	1	4
Louisiana	2	3	Washington	5	7
Maine	2	2	Washington, D. C.	13	14
Maryland	11	15	West Virginia	7	12
Massachusetts	6	12	Wisconsin	8	23
Michigan	9	14	Wyoming	0	1
Minnesota	13	18	Canada	1	14
Mississippi	2	4	France	1	1
Missouri	12	19	Italy	0	1
Montana	2	6	Porto Rico	0	1
Nebraska	3	9	Scotland	0	1
Nevada	1	1	Denmark	0	1
New Hampshire	1	2	Holland	0	1
New Jersey	11	11	Japan	0	1
New Mexico	3	1			
				355	596

President Stoltz stated that he had recommended that the finances of the association be put on a budget basis, though no definite action was taken by the Board relative to this matter.

O. E. Reed was reappointed as a delegate to the National Research Council and Dr. L. A. Rogers was reappointed as alternate.

BOARD OF DIRECTORS MEETINGS

The following action was taken by the Board of Directors at meetings on June 26 and 27. President Stoltz presided at these meetings and they were attended by C. L. Roadhouse, Vice-President, A. C. Dahlberg, Editor, R. R. Graves, Secretary, L. A. Rogers, O. F. Hunziker and Earl Weaver, Directors.

Motion adopted that the Secretary be authorized to invest another \$1500.00 in Government Bonds.

Motion adopted that the Secretary is authorized to put such additional moneys as are not needed for the current business of the association in a savings account.

The Board of Directors recommend that the Abstracts of the 1935 Annual Meeting be published by the JOURNAL in the July number; that typewritten copies of the Abstracts be submitted to the Editor by the Chairman of the Program Committee not later than the date specified by the Editor, and that a sufficient supply of page proofs be available for distribution at the Annual Meeting.

Resolved that the Editor limit all articles to 12 text pages except for articles of unusual merit.

Motion. To authorize the Secretary to secure such assistance as he may require to establish a suitable bookkeeping system for the Association, the cost not to exceed \$100.00.

Moved that the finances of the Association be put on the budget system and that for the fiscal year 1935, the budget of the JOURNAL be the income from subscriptions and advertising and an additional sum to be determined by the Board of Directors and the JOURNAL Committee.

Whereas, it is the present policy of the Association to improve the JOURNAL OF DAIRY SCIENCE and increase the membership and because the Association is now engaged in the publishing business, be it resolved that it is the policy of the Association to gradually build up a reserve of \$10,000 to cover such emergencies as may arise.

BANQUET

The banquet on Wednesday evening at the Willard Straight Hall on the campus of Cornell University was attended by all of the members present and the dining room was filled to capacity.

Professor G. F. Warren of Cornell University, who has been one of President Roosevelt's advisors on fiscal matters, spoke on the subject "Money" and it was apparent that his listeners were keenly interested. A mimeographed circular that contained graphs showing the rise and fall of commodity prices at various dates and with relation to certain historical events, helped in following Professor Warren's discussion of this intricate subject.

Dr. L. A. Rogers, Bureau of Dairy Industry, followed Professor Warren with an illustrated lecture on dairying in Italy. Doctor Rogers was an official delegate to the recent World's Dairy Congress at Rome. He told of the development of agriculture and particularly of the dairy industry in Italy. Doctor Rogers had taken motion pictures during his trip in Italy and these were shown to illustrate his talk.

THE MEETING AT GENEVA

Early on Wednesday morning, the 28th, those attending the meetings at Ithaca drove to Geneva. It was a bright clear morning after the rains of the two preceding days and this beautiful 45 mile drive was very enjoyable.

An address of welcome at the Geneva Experiment Station was given by Director U. P. Hedrick. Dr. Hedrick brought out the fact that a number of the men who have been prominent in teaching and research activities in the dairy industry of this country had been employed at the Geneva Experiment Station. Among these men were Babcock, Wing, Ladd, Van Slyke, Harding and Ward.

One of the features of the morning session at Geneva was the talk by Dr. R. S. Breed on the World's Dairy Congress in Rome. Dr. Breed had just returned from abroad and gave a very interesting talk on the work of the Congress.

Those attending the program at Geneva were the guests of W. L. Cherry, President of Cherry-Burrell Corporation; W. D. Phetepiece, President of Pfaudler Company, and of the Experiment Station at a box luncheon served on the lawn at the Experiment Station.

Just after the luncheon at Geneva Dr. Dahlberg had some of the sires and cows in the Station herd brought out on the lawn where he told of their breeding and production.

Following the afternoon session the members were entertained at a tea on the lawn of the home of Director and Mrs. U. P. Hedrick.

The Program Committee arranged the following program for the entertainment of the visiting ladies during the three days of the convention:

ENTERTAINMENT FOR LADIES

American Dairy Science Association
Ithaca and Geneva
1934

Tuesday, June 26 (Ithaca)

9-10 A.M.—Tour Willard Straight Hall and Sage Chapel.

10 A.M.-12:30 P.M.—Tour Cascadilla gorge, lake shore, and campus. Cars will be provided from Willard Straight Hall.

2-3:30 P.M.—Tour Martha Van Rensselaer Hall. Start from Willard Straight Hall.

3:30 P.M.—Picnic, Taughannock Falls State Park. Guests of Animal Husbandry and Dairy Departments. Meet at Dairy Building. Ladies will accompany the men. Transportation will be arranged for those without automobiles.

Wednesday, June 27 (Ithaca)

9 A.M.—1 P.M.—Tour Buttermilk Falls and Enfield State Parks. Start from Willard Straight Hall. Cars will be provided. Tour ends at Forest Home Inn.

1-2:30 P.M.—Luncheon, Forest Home Inn. Guests of Animal Husbandry and Dairy Departments.

4 P.M.—Tea at home of Professor and Mrs. Morrison, 315 Thurston Avenue. (Child care will be provided at home of Professor and Mrs. Guthrie, Forest Home. Supervised swimming, if desired.)

6:30 P.M.—Banquet, Willard Straight Hall. \$1.50 per plate.

Thursday, June 28 (Geneva)

8:15 A.M.—Ladies will accompany the men to Geneva. Transportation will be arranged for those without automobiles.

10 A.M.—Leave Jordan Hall for cruise on lake.

12 noon—Luncheon with the men at Experiment Station. Guests of W. L. Cherry, President of Cherry-Burrell Corporation; W. D. Phetepiece, President of Pfaudler Company; and of Experiment Station.

1:30 P.M.—Display and demonstration of making wax models. Jordan Hall.

2 P.M.—Tour Geneva and the Shuron Optical Manufacturing Company.

3:45 P.M.—Tea for every one on the lawn at the home of Director and Mrs. Hedrick.

JOURNAL OF DAIRY SCIENCE

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NUMBER 9

THE APPLICATION OF X-RAYS TO RESEARCH IN DAIRY TECHNOLOGY

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INTRODUCTION TO PROPERTIES OF X-RAYS

X-rays were discovered by Roentgen in 1895, while experimenting with cathode rays, or streams of electrons in evacuated tubes. The discoverer and certain of his contemporaries noted the similarities between x-rays and ordinary light. Both moved in straight lines, affected a photographic plate, excited fluorescence or phosphorescence in certain substances, were unaffected by magnetic fields, exhibited polarization and finally convincing proof was obtained that the velocities of the propagation of light and x-rays were identical. The essential difference between the two is in the average wave length. For crystal analysis investigation, x-rays of one Ångström unit (one Ångström unit, A.U. = 10^{-8} cm.) are used. This is about one-six-thousandth the wave length of yellow light in the visible region. Primary x-rays are generated when fast moving electrons are stopped by a target or anti cathode when sufficient potential difference between the two has been produced. Since their wave lengths λ are so much shorter, or their frequencies greater, x-rays penetrate substances which are opaque to light and are related to an intimately finer subdivision of matter than is possible for light waves.

Laue, in 1912, was the first to predict that crystals would serve as three dimensional gratings for the diffraction of x-rays, as the wave length of x-rays was of the same order of magnitude as the atomic spacing, namely 10^{-8} cm. Laue's mathematical interpretation of the patterns obtained was exceedingly complex and it remained for Bragg and Bragg to show that the crystalline structure of substances can be interpreted from the simple equation $N \lambda = 2 d \sin \theta$ when it is considered that the primary beam is reflected by the surface of the crystal. (N is a simple whole number which is the order of reflection; λ equals the wave length of x-ray beam used; d is the distance between the reflecting units; θ is equal to the angle of reflection.)

Received for publication April 30, 1934.

PURPOSE OF X-RAY ANALYSIS

The ultimate structure of crystalline material, that is, the arrangement of atoms and molecules within the crystal, and the interpretation of the properties of that crystal in terms of that arrangement is the first aim of x-ray analysis. Clark (1) states in his book *Applied X-Rays*, "the great practical importance of scientific knowledge of the ultimate structure of solids, which are crystals in their natural state, is self-evident, when consideration is given to the definition of the desired chemical and physical properties. The strength of steel girders, the corrosion of aluminum alloys, the wearing properties of case hardened steel, the plasticity of lime, the dielectric capacity of materials, the lubricating properties of long chain paraffins and graphite, the stretching of rubber, the covering power of pigments, and innumerable other practical phenomena of everyday life all depend upon ultimate crystalline structure."

Clark (1), in a general way, has listed the principal types of information that can be secured by proper interpretation of x-ray data:

1. Crystalline or non-crystalline substances
2. Crystallographic system, unit cell dimensions
3. Deduction of crystal unit (atom, ion, or molecule)
4. Chemical identity, chemical and crystallographic changes
5. Allotropic changes
6. Single crystal or aggregate
7. Type and mechanism of alloy formation
8. Random or fibered aggregate and relative degree of preferred orientation
9. Grain size in an aggregate (in colloidal range)
10. Internal strain or distortion.

Although more detailed information from x-ray analysis has been secured from substances which are commonly known to be crystalline, it has been surprising to find substances commonly thought of as being non-crystalline as actually having a partially crystalline structure and that this structure can be changed by heat treatment, pressure, stretching, etc. Casein and gelatin are examples of the latter class of substances. Stewart (2) has shown that even solutions tend to assume an orderly arrangement of groups within the solution. Hence, liquid milk should, and does show some type of arrangement (see Fig. 1). The mineral constituents and lactose are the only true crystalline constituents in dairy products that can be analyzed by x-ray, nevertheless, interesting structural changes have been observed in butterfat, milk powder, casein and cheese. The application of x-rays to research in dairy technology opens a new field of thought and endeavor.

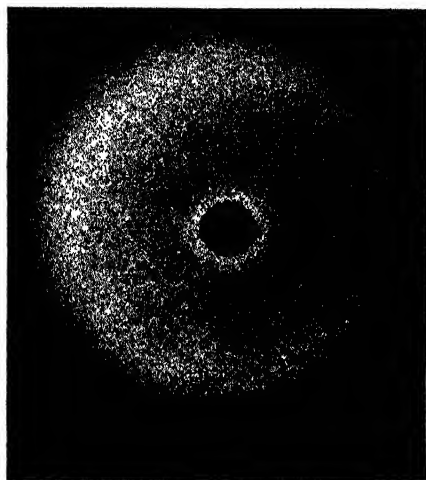


FIG. 1. LIQUID SOUR MILK

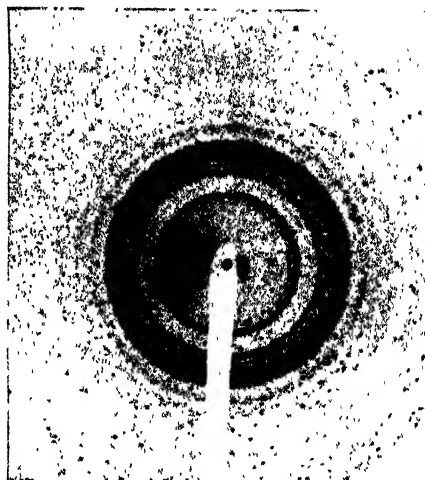


FIG. 2. POWDERED LACTOSE

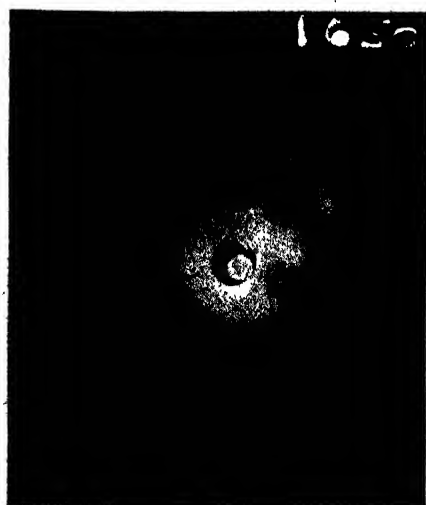


FIG. 3. DIFFRACTION SECURED BY POW-
DERED FRESH SKIM MILK—
SPRAY PROCESS

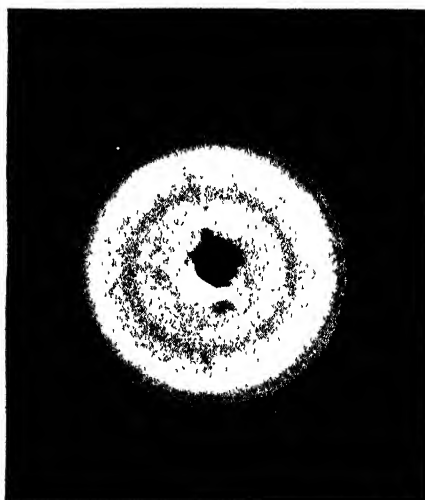


FIG. 4. POWDERED MILK IN WHICH LAC-
TOSE HAS CRYSTALLIZED

EXPERIMENTAL

This study has been of a preliminary nature in order to determine, if possible, some of the limitations and adaptations of x-rays to research in dairy products. It has served to open the field for future intensive studies in a number of directions.

The method of analysis used on the samples is known as the powder method, discovered independently by Debye and Scherrer in Europe and by Hull in the United States. The diffraction depends upon the fact that in a fine powder the particles are arranged in an entirely heterogenous manner. Since reflection occurs from a definite angle, there should be a sufficient number of particles in this powder turned at just the right angle to the primary beam of monochromatic x-rays, to enable strong reflection from one set of parallel planes; other particles turned at another angle will produce reflection from another set of planes (the same set of planes in many particles cooperating). Thus a beam passing through a powder specimen will fall upon a perpendicular flat photographic film as a series of concentric rings (Fig. 7) each of same intensity throughout and corresponding to one set of planes of spacing d .

EQUIPMENT

For this study, except in the analysis of milkstone, x-rays generated in a Müller type of tube from a copper target were used. The average wave length of the K-Alpha radiation from copper is 1.538 Ångström units. In the analysis of milkstone a General Electric tube with a molybdenum target was used. The wave length of these rays is .708 Ångström units.

ANALYSIS OF MILKSTONE

In 1930 a study of the chemical composition of milkstone was made by the authors. According to the chemical analyses, the percentage composition of milkstone varies considerably depending on its source and conditions under which it was formed. Since each chemical compound gives a definite pattern on a photographic film according to atomic arrangement, x-rays can be used for qualitative chemical analysis as well as structure analysis. When one of the samples of milkstone was analyzed by x-rays the lines which were produced were compared with the lines of several pure chemical compounds and compounds formed by the reaction of two or more compounds. When $\text{CaH}_4(\text{PO}_4)_2 + \text{Na}_2\text{CO}_3$ were allowed to react and the end product was analyzed by x-rays the lines produced compared with the sample as follows:

$\text{CaH}_4(\text{PO}_4)_2 + \text{Na}_2\text{CO}_3$													
3.5	2.84	2.30	2.31	1.98	1.87	1.77	1.73	1.465	1.328	1.252	1.16	1.12	1.038 .985
Milkstone													
3.5	2.86	2.64	2.31	1.97	1.84			1.735	1.46		1.25	1.16	1.12 1.033 .96

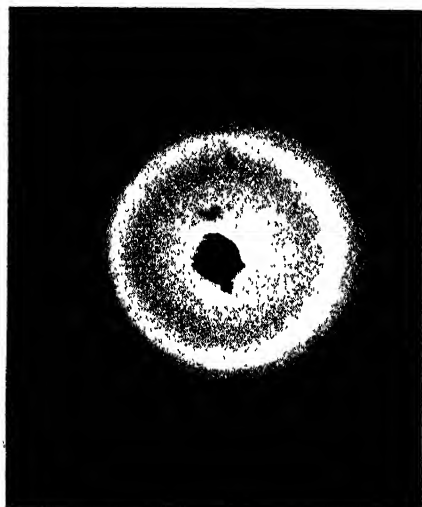


FIG. 5. LACTOSE BEGINNING TO CRYSTALLIZE IN SKIM MILK POWDER

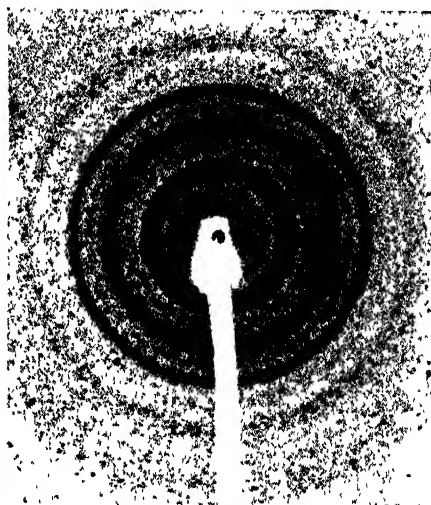


FIG. 6. SUCROSE

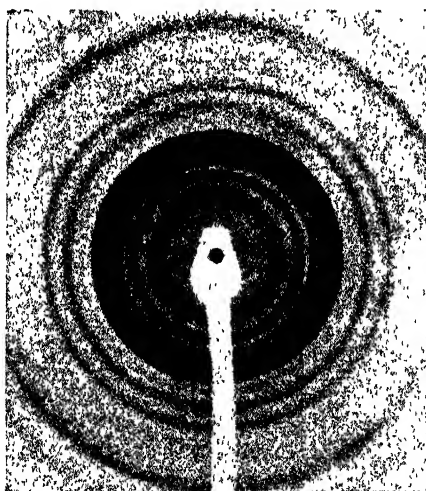


FIG. 7. DEXTROSE

There is a possibility for the reaction to proceed in two ways: First a double decomposition reaction to form $\text{CaCO}_3 + \text{Na}_2\text{H}_4(\text{PO}_4)_2$; or second, to form $\text{CaHPO}_4 + \text{Na}_2\text{HPO}_4 + \text{CO}_2 + \text{H}_2\text{O}$. The latter reaction seems to be the most likely since no carbonate (CO_2) was found in the end product.

THE ANALYSIS OF MILK POWDER

Thus far, in the study of milk powder, most of the work has been confined to determining the effect of the different milk powdering processes upon the structural group spacings within the milk proteins. Since the units that diffract x-rays are much farther apart in proteins than in inorganic materials, then x-rays of longer wave length than those used for inorganic crystal analysis should be used. However, the propagation and handling of x-rays of long wave length have not been carried beyond the experimental stage. Hence x-rays from copper, although much longer than those used for steel analysis are still shorter than is desired for protein diffraction analysis. However, much information still can be secured by the use of x-rays of this length from copper.

Although structural changes within the milk protein due to different types of processing equipment are not marked, there is a tendency for a shrinkage in unit spacing with an increase in heat treatment. Hence milk powders made by the roller process have a tendency for a smaller d_1 unit spacing than do milk powders of the spray types. This is illustrated by the following measurements made on the different types:

<i>Spray Process</i>	<i>Vacuum Roll</i>	<i>Atmospheric Roll</i>
1a. $d_1 = 4.63$	1b. $d_1 = 4.625$	1c. $d_1 = 4.258$
2a. $d_1 = 4.63$	2b. $d_1 = 4.60$	2c. $d_1 = 4.473$
3a. $d_1 = 4.625$		3c. $d_1 = 4.473$
4a. $d_1 = 4.616$		4c. $d_1 = 4.493$
5a. $d_1 = 4.596$		5c. $d_1 = 4.495$
6a. $d_1 = 4.593$		6c. $d_1 = 4.545$
7a. $d_1 = 4.593$		7c. $d_1 = 4.588$
8a. $d_1 = 4.588$		
9a. $d_1 = 4.587$		
10a. $d_1 = 4.51$		

Further evidence of this is secured by the measurements secured on scorched milk powder as compared with a normal sample.

<i>Scorched Roller Powder</i>	<i>Normal Spray Powder</i>
1. Fairly scorched powder $d_1 = 4.48$	1a. $d_1 = 4.63$
2. Scorched powder $d_1 = 4.484$	
3. Badly scorched powder $d_1 = 4.473$	

The lines secured from the analysis of milk powder are due entirely to the diffraction of x-rays by units within the protein molecule, although lac-

tose constitutes approximately 50 per cent of skim milk powder. This fact indicates that lactose is not in a crystalline state in normal milk powder, but may be in one of two conditions; namely minute crystals absorbed on the protein and in too small units to diffract x-rays or it may be in an amorphous or glass state. The former is more probable since a larger halo on the film would be more likely if the lactose were in an amorphous condition. As soon as moisture is absorbed from the air the lactose crystallizes within the milk powder. This is illustrated by Fig. 4. (Compare Figs. 2 and 4.) No protein lines are in evidence although this sample is of the same lot as shown in Fig. 3. The only difference is in the size and state of crystal structure of the lactose. Troy and Sharp (3) have pointed out that the caking of milk powder is due to the absorption of moisture followed by the crystallization of lactose. Their experimental work was based upon observations with a polarizing microscope. However, x-rays show the very beginning of crystallization as illustrated by Fig. 5. Crystallization at this stage could not be shown with a polarizing microscope and can only be shown by x-ray analysis.

W. T. Ashbury (4) claims that the lines of proteins are due to the diffraction of x-rays by the amino acid residues in the protein molecule. He gives 3.5 A.U. for the amino acid residue in natural silk (fibrous), 3.4 A.U. for stretched hair (B-keratin) and 3.3 A.U. for stretched feather keratin. Trillat (5) gives the spacing for gelatin as follows: d 4.3; d_1 15.9; d_2 2.8.

DIFFERENTIATION OF SUGAR

Since each crystalline compound gives a definite pattern according to atomic arrangement, the identification and differentiation of the common sugars (sucrose, dextrose and lactose) is made simple by x-rays as illustrated by Figs. 6, 7, and 2.

POSSIBILITIES OF FURTHER STUDY

Although the work reported has been confined to the two subjects mentioned, there are a number of problems suggested by this study that may be investigated.

The structural changes in casein during cheese ripening at once presents itself as an attractive problem. A study of the changes occurring in butterfat also lends itself to analysis by x-rays. The arrangement and size of space groups of lactose have not yet been reported, altho this has been worked out for the other sugars. The quantitative determinations of particle size of ice crystals in ice cream also can be determined with the aid of x-rays.

SUMMARY

By x-rays analysis the identification of the mineral constituents in a sample of milkstone was determined. Results obtained from the diffraction

of x-rays by milk powder show conclusively that lactose does not exist in a crystalline form in fresh milk powder. The very onset of lactose crystallization in milk powder was shown by the diffraction patterns obtained. Also a tendency for a change of unit spacing of the diffracting group in milk protein due to different processing methods has been noted. Spray powders were found to have a slightly greater d_1 unit spacing than the roller powders.

REFERENCES

- (1) CLARK, G. L. Applied X-Rays, 2nd Edition McGraw-Hill, p. 183. 1932.
- (2) *Ibid.* p. 433.
- (3) TROY, H., AND SHARP, PAUL F. JOUR. DAIRY SCI. 13: (2). 1930.
- (4) ASHBURY, W. T. Transactions of the Faraday Society, No. 140, Vol. XXIX, Part I. January 1933.
- (5) TRILLAT, T. J. Journal de Chemie Physiques. 25. January 1932.

SOME PHYSICO-CHEMICAL PROPERTIES OF LACTOSE

III. THE AQUEOUS VAPOR TENSION OF ALPHA HYDRATE- ANHYDRIDE SYSTEMS. THE PREPARATION OF ALPHA ANHYDRIDE

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INTRODUCTION

The reliability of such constants as the specific rotation, the heat of combustion, or the reducing power of lactose is dependent upon the accuracy with which the moisture content of the material is known. There is need for information regarding conditions under which lactose can be dried superficially without loss of water of crystallization, as well as regarding the conditions under which the anhydride itself may be prepared with a minimum of decomposition.

A study of the literature revealed such conflicting statements that it seemed desirable to gather more data in order to determine these conditions, and to discover, if possible, the cause of the discrepancies which were found.

Hudson (7) is apparently the only one who has attempted to determine the dissociation pressure of lactose hydrate. His values range from 73 mm. of mercury at 60° C. to 433 mm. of mercury at 90° C. He proposed the formula:

$$\log p = 10.2176 - 3115/T$$

for interpolation. This gives a value of 735 mm. of mercury at 100° C. From these results, we must conclude that lactose hydrate is unstable even at 50° C., when in contact with the atmosphere. On the other hand, it has been believed by many workers that the hydrate could be heated to 100° C., without danger of losing water of crystallization. In 1856 Lieben (9) stated that lactose began to lose its water of hydration at 110° C. Schmoeger (11) in 1880 wrote, "Air dry, or sulphuric acid dried crystallized lactose $C_{12}H_{22}O_{11} + H_2O$, does not change in weight during several hours drying at 100° C., as is also reported by other writers." He republished (12) a similar statement in 1881. In 1896, Tanret (13) reported that crystallized lactose was not dehydrated at 100° C. Holty (4), who determined the specific rotation of lactose in pyridine, dried the hydrate at 100° C. to free it of "excess moisture." In 1912, Browne (1) stated that lactose may be dried at 100° C., and then weighed as the hydrate. In

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1926, Rice and Miscall (10) stated, "It is known that lactose hydrate is very stable, the molecule of water being driven off only at about 130° C." Then in 1928, Verschuur (14) stated that while lactose lost its moisture below 125° C., the loss was very slow.

If Hudson's data are correct, then it is evident that much of the data of other workers is subject to suspicion. In any case, both the work of Hudson and of others should be repeated and either confirmed or proven in error.

Careful consideration of Hudson's data places it under suspicion at once. Vapor pressure measurements are meaningless unless both the hydrated form and the anhydrous form are present in contact with water vapor. Hudson's vapor pressure measurements were made upon a system of lactose hydrate and beta anhydride. There is no evidence whatever that the hydrated form of alpha lactose can be broken down by heat into water and beta anhydride, although both Hudson (7) and Gillis (3) have attempted to accomplish it. This unfortunate choice of components of his system doubtless came about through Hudson's view (5, 6, 8) that hydrated lactose is neither the alpha nor the beta form, but is the hydrated aldehyde and is related equally to both beta and alpha anhydrides.

Since the components selected by Hudson do not represent a reversible system, he might have measured any pressure higher than the true pressure, but it is improbable that his measurements would be too low. His measurements would not show constant values unless a reversible system were formed by the decomposition of some of the lactose. That this actually occurred is probable since he found it necessary to heat for several weeks in order to obtain constant vapor pressures. Lactose is known to break down rather easily at temperatures of 130° C., and probably decomposes slowly at much lower temperatures. Moreover, only a very small amount of decomposition would be necessary to account for the observed high pressures. The only alternative to this conclusion would be for lactose to have a very high vapor pressure at equilibrium, but an extremely slow rate of loss or gain of moisture.

If the rate of loss of moisture from lactose is as slow as suggested, then data on equilibrium pressures alone would not be of great practical value. It would be necessary to know not only at what temperatures lactose can lose water, but also at what temperatures the loss would be sufficiently rapid to be of use. For that reason, experiments on vapor pressure were of two kinds. One was directed toward measurement of the rates at which water was lost or gained; the other, toward a study of equilibrium pressures.

EXPERIMENTS

The rate at which lactose loses water depends upon at least the three factors: temperature, size of crystals, and the moisture content of the at-

mosphere. In these experiments, control of the humidity was not attempted. Lactose crystals of different sizes were placed in small aluminum dishes and held in an electric oven at temperatures of 100° C., 80° C., and 70° C. In order to secure greater uniformity of temperatures among the dishes, a disk of aluminum $\frac{1}{4}$ inch thick was placed under the dishes. The thermometer used to regulate the temperature had its bulb immersed in a separate dish of lactose. The lactose samples were prepared by a careful sifting of pure lactose in a mechanical shaker. The samples may be described as follows:

- A Impalpable powder, a commercial product.
- B Lactose passing a 200 mesh screen (200 spaces per inch).
- C Lactose passing a 100 mesh screen, but retained on 200 mesh.
- D Lactose passing an 80 mesh screen, but retained on 100 mesh.
- E Lactose passing a 40 mesh screen, but retained on 80 mesh.
- F Lactose passing a 20 mesh screen, but retained on 40 mesh.

Two-gram samples were weighed carefully into the dishes. After suitable intervals in the drying oven, the dishes were removed quickly, covered, cooled, and weighed. They were then returned to the oven. The per cent of loss in weight is plotted against time in figures 1 and 2.

The results of these experiments show that lactose does lose its water of crystallization at 100° C., or even at 80° C., in an air oven, but that the rate of loss varies greatly with the size of the crystals. This probably accounts for Gillis' remark (3, page 91): "*Il est absolument nécessaire de se rendre compte par pesée que le lactose est entièrement déshydraté, car la vitesse de déshydratation varie notablement d'une préparation à l'autre.*" The influence of the size of the crystals was demonstrated strikingly by the fact that a single well formed crystal of lactose weighing 0.4183 grams lost only 0.0012 grams after 92 hours in a Mojonnier oven at 100° C., under 25 inches of vacuum.

The more finely divided samples of lactose gave much smoother "loss in weight" curves than the coarser samples. This is due to the fact that it is quite possible to remove all large crystals from the finer samples, but impossible to remove all of the fine crystals from the coarser samples. A sharp separation was made more difficult by the fact that some of the particles in the larger sizes were not single crystals but were clusters of small ones.

All of the samples show an initial loss followed by a period during which the rate of loss was very slow. This is doubtless due to the fact that crystals may often be heated for some time without appreciable loss of water until they become seeded with the anhydrous modification. As soon as nuclei of the anhydrous form appear, the loss of water takes place normally (2). This phenomenon probably accounts for the failure of the large crystal to lose water in the experiment reported above.

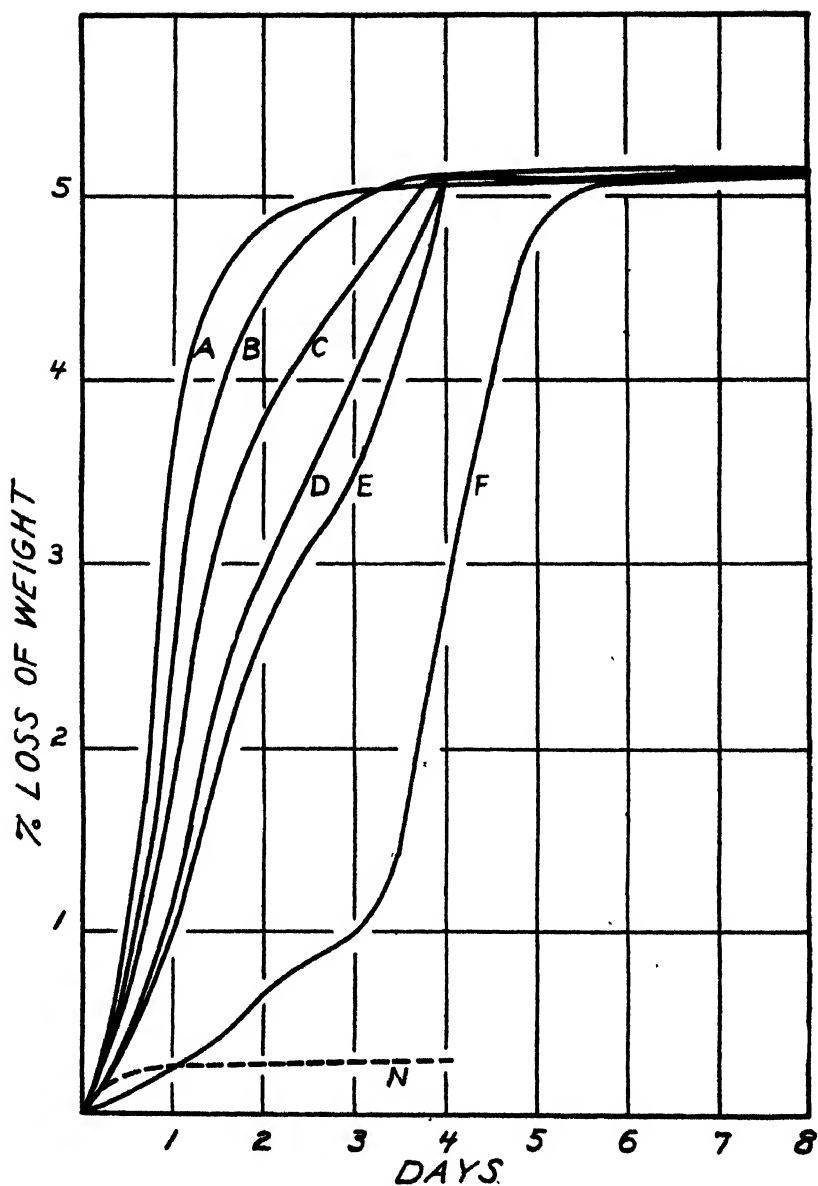


FIG. 1. The rate of moisture loss from alpha hydrate at 100° C., in an air oven. A study of crystals of six different sizes. Dotted line, N, a single large crystal held at 100° C., in vacuo.

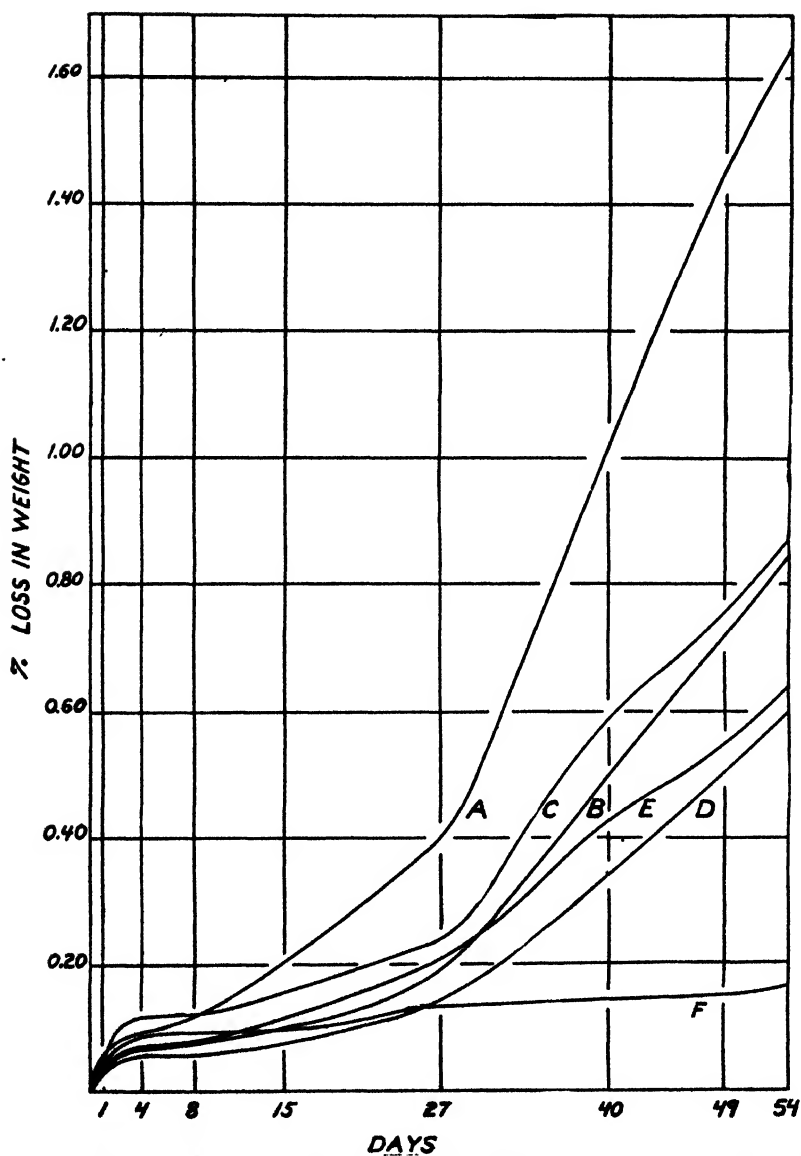


FIG. 2. The rate of moisture loss from alpha hydrate at 80° C., in an air oven. A study of crystals of six different sizes.

The data show that the loss of water may be quite rapid at 100° C., and quite appreciable even at 80° C. After holding the samples for 54 days at 80° C., the temperature of the oven was lowered to 70° C. At this temperature, the loss in weight was almost nil, averaging 0.02 per cent after 35 days. It should be remembered that these samples were heavily seeded with the anhydrous form.

The ease with which equilibrium of vapor pressure is established depends upon the rate at which water is taken up, as well as upon the rate at which it is lost. Experience has indicated that alpha anhydride takes up moisture quite rapidly. However, an experiment was carried out to determine how rapidly moisture is taken up, and how much the rate of absorption is influenced by the size of the crystals. One-gram samples of lactose were weighed into aluminum dishes, and dried in a vacuum oven at 100° C. Each dish was then placed upon a balance, and a beaker of water was placed in the case. The sliding door was left raised about one inch. The dishes were weighed periodically, and the increases in weight are shown in table 1.

TABLE 1
The rate of absorption of moisture by alpha anhydride crystals of different sizes

TIME IN MINUTES	MOISTURE CONTENT, PER CENT	
	Sample C	Sample E
0	0	0
10	.62	.52
20	1.24	1.14
40	2.36	2.31
60	3.27	3.32
80	3.95	4.03
120	4.75	4.79
160	5.08	5.10
200	5.19	5.15
280	5.31	5.25

Sample C: from hydrated lactose passing a 100 mesh screen but retained on a 200 mesh screen.

Sample E: from hydrated lactose passing a 40 mesh screen but retained on an 80 mesh screen.

The values given are averages of duplicate determinations.

The results indicate no difference due to crystal size. The limiting factor seemed to be the rate of diffusion of water vapor to the sample.

An attempt was made to measure the vapor pressure of lactose hydrate-anhydride systems. A sample of lactose passing a 100 mesh screen, but retained by a 200 mesh screen, was placed in a tensiometer. Phosphoric anhydride was placed in the other arm of the tensiometer, and mercury was placed in the manometer. The apparatus was carefully evacuated, by means of a Sprengel pump, and then sealed. In constructing the tensiometer

eters, care was taken that the two arms were approximately of equal volumes. The tensiometers were mounted in a glass walled thermostat, and the readings were made with the aid of a cathetometer. Corrections were not made for variation in the specific gravity of the mercury. The thermostat was provided with an efficient circulating device, and temperature fluctuations were always less than 0.10° C. In some of the experiments, the water bath was replaced by a liter beaker of boiling water. Pumice stone was used to prevent superheating, and enough heat was supplied to keep the water boiling vigorously.

It was soon found that accurate measurement of the vapor pressure of lactose was a more difficult problem than had been supposed. After the tensiometer had been immersed in the heated bath, the pressure rose rapidly for a few minutes and then showed a slow drift toward higher values. This drift was scarcely noticeable at 50° C., but was very pronounced at 100° C. Table 2 shows the data obtained in one experiment at 99° C. The

TABLE 2
The rate of pressure rise in lactose hydrate-anhydride systems held at 99° C.

TIME IN MINUTES	PRESSURE IN MM. OF Hg	RATE OF RISE MM. PER MINUTE
0	0	
10	26.1	2.610
20	27.8	0.170
30	28.3	0.050
100	29.9	0.023
160	30.2	0.005
280	31.6	0.012
400	32.3	0.006
520	33.5	0.010
640	34.2	0.006
985	36.7	0.007

tensiometer used had not been heated previously. It was immersed in a large beaker of boiling water, and measurements of vapor pressure were made at regular intervals. The table shows that the rate of pressure development was high at first, and then decreased to an almost constant value. It is believed that the first rapid rise represented the actual vapor pressure of the lactose while the slower increase which followed was due to some other unknown transformation. In order to determine the position of the break in the curve, the rates of pressure increase in mm. per minute were calculated, and are shown in the third column of the table. The rate was found to reach a minimum value at 160 minutes, and the pressure recorded at that time was arbitrarily taken as the vapor pressure of alpha hydrate in contact with its anhydride.

Table 3 shows the pressures which were obtained at various temperatures. They are offered as approximations only, because of the difficulty

in determining the break in the curve at the higher temperatures. However, it is believed that they are not greatly in error.

TABLE 3
*The approximate vapor pressure of lactose hydrate-anhydride systems
at various temperatures*

TEMPERATURE ° C.	PRESSURE IN MM. OF HG.
52.0	1.23
57.2	2.3
76.1	8.3
84.0	13.4
93.5	23.1
99.0	30.2

It would not be permissible to dismiss the gradual drift toward higher pressures as due to decomposition, without additional evidence. Previous experiments have shown that regardless of the size of crystals, the absorption of water vapor by the anhydride is exceedingly rapid, governed apparently by the rate of diffusion of water vapor. However, it was found that when the tensiometers were cooled the pressure did not fall to zero at once, but fell to a few mms. pressure and then decreased very slowly. If the tensiometer had been heated for several days, the pressure sometimes did not return to zero. This slow drift on cooling is shown in table 4, which is really a continuation of table 2. After 985 minutes at 99° C., the tensiometer was cooled to 25° C., and the pressure measurements were made at the intervals shown in the table.

A study of the data suggested that two processes were taking place. One, a fairly rapid reaction was responsible for the initial rise in pressure. The second was very much slower, and gave rise to the slow drift when

TABLE 4
*The rate of pressure fall in lactose hydrate-anhydrate systems when cooled
quickly from 99° to 25° C.*

TIME IN MINUTES	PRESSURE IN MM. OF HG.	RATE OF FALL MM. PER MINUTE
0	36.7,	0.0
15	3.3	2.23
30	2.7	0.40
4305	1.4	0.00030
5745	0.9	0.00035

the temperature was either raised or lowered. If this were actually the case, it should be possible to demonstrate the existence of two equilibria which are established at different rates.

Let it be assumed that reaction A is quite rapid, and reaction B is slow. Let us further assume that a given temperature, the sum of the two pressures is unity. If the system is cooled quickly, then a new pressure will be indicated whose magnitude would depend upon the pressure of system A at the lower temperature, and the pressure of system B at the original higher temperature. If the apparatus is quickly heated to the original temperature, the original pressure of unity should be established since we have assumed that system A is able to adjust itself rapidly, and that there is no shift in system B during the initial cooling. If, however, the apparatus is held at the lower temperature long enough for the slower equilibrium B to adjust itself, then rapid heating to the original temperature would not restore the original pressure. Time would be required for the slower process to come to equilibrium. These predictions were tested experimentally and verified.

The tests were made with a tensiometer which had been in use for some time, and at the start of the experiment it still showed a residual pressure from previous heating. It was placed in a bath of boiling water and pressure readings were taken until the slow drift appeared. Then the tensiometer was lifted from the bath and allowed to cool in the air for exactly one minute. It was then returned to the water bath and the observations were continued. This was repeated a second time. Finally, at the end of the experiment, the apparatus was cooled to room temperature and a few observations were made upon the drift again. These data are shown in table 5. They agree very well with the behavior predicted for a system made up of two independent reactions, one rapid, the other slow. Originally, it required thirty minutes for the pressure to rise from 35.6 mm. to 39.1 mm., but after cooling until the pressure fell to 15 mm., only a five-minute heating period was required to establish a pressure of 39.8 mm., a pressure even higher than the initial one. When the cooling was repeated, similar results were obtained.

In order to complete the proof that there must be two equilibria, it must be shown that if the tensiometer is held at a low temperature for a considerable period of time, the original high pressure would not be reestablished on heating until the slower reaction had time to adjust itself to the new temperature. The truth of this assumption was demonstrated by experiment. The data are shown in table 6. The same apparatus used for the experiment of table 4 was reheated in exactly the same way as before. This tensiometer had previously registered a maximum pressure of 36.7 mm., and at the beginning of this experiment it still showed a residual pressure of 0.5 mm. As was to be expected, the pressure rose to higher values

TABLE 5

The effect of momentary cooling on the rate of pressure rise in a lactose hydrate-anhydride system at 99° C.

TIME IN MINUTES	PRESSURE IN MM. OF Hg.	REMARKS
0	1.7	Residual pressure
5	35.6	
10	37.1	
15	37.4	
35	39.1	
1	15.0	Held in the air at 28° C.
5	39.8	Returned to water bath at 99° C.
10	40.7	
15	41.1	
1	17.0	Held in the air at 28° C.
5	40.1	Returned to water bath at 99° C.
10	40.3	
5	5.0	Held in water at 25° C.
10	3.9	
15	3.6	
30	3.5	

This experiment was performed without interruption. The time in minutes measures the period under each treatment.

during the first few minutes of heating than had been obtained during the initial run. But the pressure did not rise at once to the previous maximum value. The experiment was not continued until that pressure was reached, but the evidence shows that a long drift period would be required.

TABLE 6

The effect of holding six days at room temperature on the rate of pressure rise in a lactose hydrate-anhydride system at 99° C.

TIME IN MINUTES	PRESSURE IN MM. OF Hg.
0	0.5
5	29.4
10	30.7
15	31.1
20	31.4
30	31.7
50	32.5
60	32.9

SUMMARY

Hydrated lactose has been found to lose its water of crystallization at temperatures as low as 80° C., when heated in an air oven, but the rate of loss is very slow. The rate is very much greater from small crystals than from large ones. However, the crystal size is of little importance in determining the rate at which moisture is taken up by the anhydride.

Measurements of the vapor pressure of the system alpha hydrate-anhydride were made at temperatures between 50° C., and 100° C. Evidence is presented to show that some reaction other than loss of water of hydration must occur on continued heating of lactose. The loss of water is considered to be a relatively rapid process. The unknown reaction is very much slower. The pressures measured by Hudson which are approximately twenty times those reported here, probably are due to the slower equilibrium whose nature is unknown.

REFERENCES

- (1) BROWNE, C. A. A handbook of sugar analysis. 1st edition, p. 25. John Wiley and Sons, New York. 1912.
- (2) FINDLAY, ALEXANDER. The phase rule and its applications. 6th edition, p. 84. Longmans Green and Company, London. 1927.
- (3) GILLIS, J. Nouvelles recherches sur le sucre de lait. *Rec. trav. chim.* **39**: 88-125. 1920.
- (4) HOLTY, JOSEPH GERARD. Solubility and specific rotatory power of carbohydrates and certain organic acids and bases in pyridine and other solvents. *J. Phys. Chem.* **9**: 764-79. 1905.
- (5) HUDSON, C. S. Über die Multirotation des Milchezuckers. *Z. physik. Chem.* **44**: 487-94. 1903.
- (6) HUDSON, C. S. The hydration of milk sugar in solution. *J. Am. Chem. Soc.* **26**: 1065-82. 1904.
- (7) HUDSON, C. S. Further studies on milk sugar. *J. Am. Chem. Soc.* **30**: 1767-83. 1908.
- (8) HUDSON, C. S. A review of discoveries on the maturation of sugars. *J. Am. Chem. Soc.* **32**: 889-94. 1910.
- (9) LIEBEN. Abstracted in *Jahresber. Chem.* (1856): 646.
- (10) RICE, F. E., AND MISCALL, JACK. Sweetened condensed milk. III. In a total solids residue, what is the form of lactose? *JOUR. DAIRY SCI.* **9**: 62-4. 1926.
- (11) SCHMOEGER, M. Eine bis jetzt noch nicht beobachtete Eigenschaft des Milchezuckers. *Ber.* **13**: 1915-21. 1880.
- (12) SCHMOEGER, M. Ueber wasserfreien Milchezucker. *Ber.* **14**: 2121-6. 1881.
- (13) TANRET, C. Sur les modifications moléculaires et la multarotation des sucres. *Bull. soc. chim.* **15**: 349-61. 1896.
- (14) VERSCHUUR, R. Le changement de la rotation et du pouvoir conducteur de quelques sucres en solution aqueuse avec et sans acide borique. *Rec. trav. chim.* **47**: 123-52. 1928.

IRRADIATED MILK: THE REFLECTING PROPERTIES AND ANTIRACHITIC ACTIVATION AS AFFECTED BY THE IMPINGEMENT ANGLE OF THE INCIDENT RADIATION

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The relationship between the character and intensity of the ultra-violet radiation applied to milk films and the antirachitic potency imparted to the milk during momentary periods of exposure has been previously reported (1-4). Milk films 0.11 mm. thick transmit less than 5 per cent of the radiation below 2850 Å incident to the surface at an angle of 90° (2). Since the incident radiation may be transmitted, absorbed or reflected, the difference between the amount of energy applied and that transmitted does not necessarily indicate the amount absorbed. According to Drothus' law only absorbed radiation can cause photochemical change. It does not necessarily follow, however, that all the energy absorbed brings about photochemical reactions; much of the absorbed energy may be manifested as heat. If data are to be developed which will more adequately show the character and the amount of radiant energy necessary for the most effective and efficient antirachitic activation of milk, it is necessary to know the degree to which the activating rays are reflected from milk surfaces. Such data are recorded in the present paper.

EXPERIMENTAL

Vertical flowing milk films of known characteristics were formed with the apparatus described in an earlier paper (2). The carbon arc burning C type electrodes at 60 amperes and 50 volts was used for supplying the beam of ultra-violet radiation. The spectroradiometer previously used was placed in such a position that a beam of the radiation reflected from the milk surface was reflected into the instrument. The amount of radiation received by the spectroradiometer compared with that received by the instrument placed in such a position that it received the same beam from the same source at the same optical distance without the intervening milk film, permitted the calculation of the percentage of energy reflected. Spectral or mirror reflection from the surface of flowing milk films receiving the radiation at different angles of incidence is shown by the curves in Chart I. (The angle of incidence as referred to in this paper is to be interpreted as

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the angle between the surface of the milk and the incident radiation; a beam parallel to the milk surface being zero degrees and a beam intersecting the milk at right angles being indicated as 90° .) In presenting these data it is recognized that in the visible portion of the spectrum there would be a certain degree of diffuse reflection originating at some distance below the

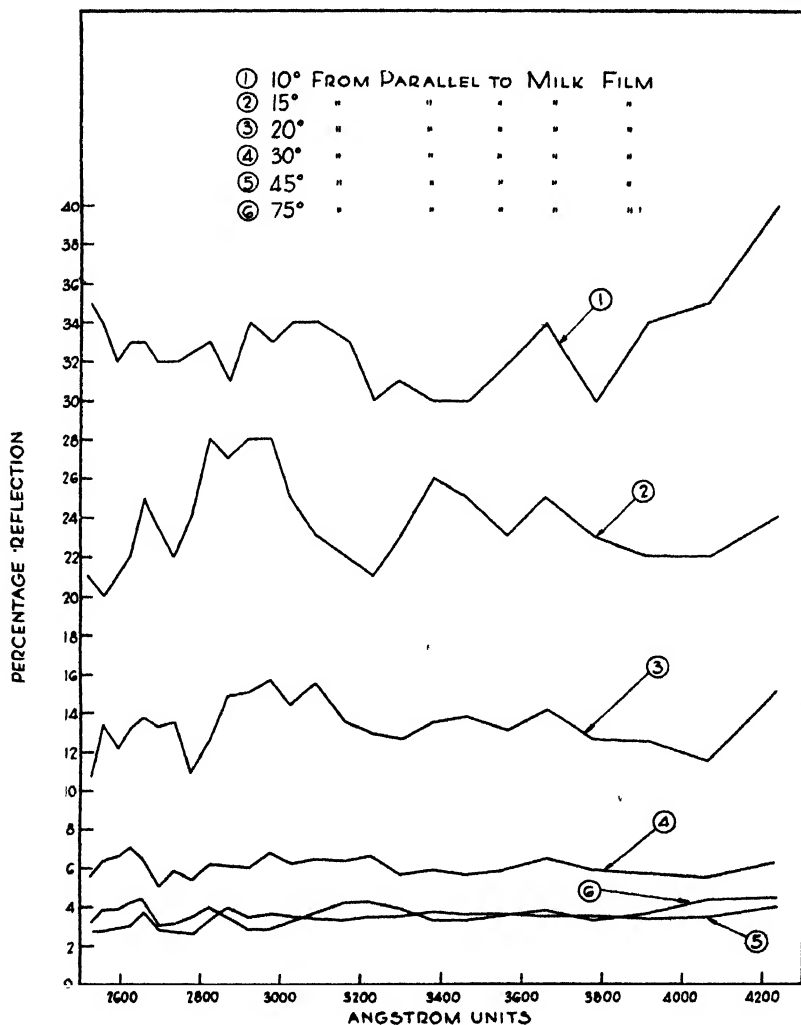


CHART I

The reflection of ultra-violet radiation from the surface of milk.

immediate surface of the milk. However, in view of the transmission data previously obtained, and in view of the results shown by preliminary measurements, wherein it was found that diffuse reflection of the wave lengths under consideration were too weak to be measured with the apparatus

available, it is believed that the effect of this factor has been slight or even negligible in the results obtained.

The reflection measurements shown by the graphs are the average of three separate determinations from whole milk films 0.11 mm. thick. Similar measurements from skimmed milk films were made for the 15° and 30° angle of incidence. The results showed that throughout the 2550–3000 Å range the reflection from skim milk, although tending to parallel that of whole milk was from 1 to 2 per cent lower between 2550 and 2800 Å, and from 2 to 8 per cent lower in the 2800 to 3000 Å region. The reflection from the milk films compared with the reflection from the surface of water, calculated for the range of wave lengths and angles of incidence under consideration, shows that the reflection from both substances is of the same order of magnitude. However, the calculated reflection from water, as well as actual measurements from vertical flowing water films, shows that the water surface does not exhibit the selective reflection shown by milk. Since the transmission data has indicated a selective absorption of ultra-violet radiation by milk, selective reflection may be expected.

In order to determine the possible significance of the reflection of ultra-violet radiation from milk surfaces on the antirachitic potency, milk films of known thickness and flow characteristics were irradiated by radiation of known intensity impinging at different angles of incidence. The carbon arc of the same characteristics as was used for obtaining the reflection data was used for irradiating the milk films. The arc was placed 43 cm. from the center of the milk films having a width of 10 cm. The films were formed on the flow board mounted on a vertical central axis which permitted accurate change of the plane of flow with reference to the projected beam of radiation. Milks containing 1.2 per cent and 3.6 per cent butter fat were used. The biological assays were made by the usual methods, and the results recorded in the manner heretofore used (1–5). These results as well as those showing the amount of energy applied per cc. of milk are recorded in table 1. In determining the amount of energy applied to the milk film, the effect of the difference in angle of impingement was taken into consideration. Since the data do not purport to show the amount of energy absorbed, that which was reflected has not been considered in the calculations. However, an approximation of the amount of energy absorbed may be calculated from the transmission data (2) in conjunction with that shown herein. An analysis of the reflection data shows that at an impingement angle of 75° from parallel, an average of 3.1 per cent of the incident radiation between 2550 and 3000 Å is reflected; at an impingement angle of 45° from parallel an average of 2.9 per cent is reflected; at 30° , the average is 6.1 per cent; at 20° , 13.4 per cent is reflected; at 15° , 28 per cent; and at 10° , 34 per cent.

The biological results show that the reduction in intensity of the incident radiation resulting from impinging the energy at angles of incidence as low as 30° from parallel does not cause a reduction in antirachitic potency of the milk treated under the conditions used in these tests. At impingement angles of 15° there is definite evidence of a reduction in anti-

TABLE 1

The vitamin D concentration of milk as affected by film thickness, film capacity, fat content, and angle of impingement of incident radiations (2000-3000 Å)

SAMPLE	TEST SUBSTANCE	MILK FILM CHARACTERISTICS			EXPOSURE PERIOD	ANGLE OF INCIDENCE	TOTAL ERGS PER CC. (× 10 ⁴)	TOTAL QUANTA PER CC. (× 10 ¹⁴)	TOTAL MILK FED cc.	VITAMIN D PER CC. (× 10 ¹⁰)
		Capacity per inch per minute	Thickness	Vertical distance of travel dur- ing exposure						
		<i>gms.</i>	<i>mm.</i>	<i>cm.</i>	<i>secs.</i>	<i>degs.</i>				<i>mols.</i>
1A190	Milk, 1.2% Fat	2.12	0.02	0.66	1.0	90	8,945	11,851	60	75
1A175	" " "	"	"	"	"	75	8,640	11,446	—	—
1A160	" " "	"	"	"	"	60	7,746	10,103	60	75
1A145	" " "	"	"	"	"	45	6,325	8,379	60	75
1A130	" " "	"	"	"	"	30	4,472	5,925	60	75
1A115	" " "	"	"	"	"	15	2,282	3,067	60	75
3A190	Milk, 1.2% Fat	34.02	0.11	40.64	1.87	90	3,769	4,994	20	225
3A175	" " "	"	"	"	"	75	3,640	4,823	20	225
3A160	" " "	"	"	"	"	60	3,284	4,325	20	225
3A145	" " "	"	"	"	"	45	2,665	3,531	20	225
3A130	" " "	"	"	"	"	30	1,884	2,497	60	75
3A115	" " "	"	"	"	"	15	975	1,292	60	75
6A190	Milk, 1.2% Fat	192.78	0.23	40.64	0.89	90	972	1,288	50	90
6A175	" " "	"	"	"	"	75	939	1,244	—	—
6A160	" " "	"	"	"	"	60	841	1,115	—	—
6A145	" " "	"	"	"	"	45	687	910	50	90
6A130	" " "	"	"	"	"	30	486	644	—	—
6A115	" " "	"	"	"	"	15	123	333	80	56
1AW190	Milk, 3.6% Fat	2.12	0.02	0.66	1.0	90	8,945	11,851	30	150
1AW175	" " "	"	"	"	"	75	8,640	11,446	—	—
1AW160	" " "	"	"	"	"	60	7,746	10,103	30	150
1AW145	" " "	"	"	"	"	45	6,325	8,379	30	150
1AW130	" " "	"	"	"	"	30	4,472	5,925	30	150
1AW115	" " "	"	"	"	"	15	2,282	3,067	70	64
3AW190	Milk, 3.6% Fat	34.02	0.11	40.64	1.87	90	3,769	4,994	20	225
3AW175	" " "	"	"	"	"	75	3,640	4,823	20	225
3AW160	" " "	"	"	"	"	60	3,284	4,325	20	225
3AW145	" " "	"	"	"	"	45	2,665	3,531	20	225
3AW130	" " "	"	"	"	"	30	1,884	2,497	20	225
3AW115	" " "	"	"	"	"	15	975	1,292	30	150
6AW190	Milk, 3.6% Fat	192.78	0.23	40.64	0.89	90	972	1,288	35	128
6AW175	" " "	"	"	"	"	75	939	1,244	35	128
6AW160	" " "	"	"	"	"	60	841	1,115	35	128
6AW145	" " "	"	"	"	"	45	687	910	35	128
6AW130	" " "	"	"	"	"	30	486	644	35	128
6AW115	" " "	"	"	"	"	15	123	333	50	90

rachitic potency. The data as a whole are considered as further confirmatory evidence supporting the conclusion that the synthesis of Vitamin D during the irradiation of milk is essentially a surface reaction and that in the practical treatment of milk with ultra-violet rays, conditions which facilitate the reaction at the surface are to be preferred.

SUMMARY

1. The reflection of ultra-violet radiation from the surface of milk films is of the same order of magnitude as the reflection of such radiation from the surface of water; the degree of reflection increases as the angle of incidence decreases.

2. Ultra-violet radiation between 2550 and 3000 Å when impinged on milk surfaces at 75° and 45° from parallel are reflected to about the same degree—approximately 3 per cent; the average percentage reflection from a 30° angle of incidence is about 6 per cent; from a 20° angle of incidence, 13.4 per cent; from a 15° angle, 28 per cent; and from a 10° angle, about 34 per cent.

3. Milk films show the property of selective reflection of ultra-violet radiation, especially throughout the range 2550 to 3300 Å. This property is most pronounced at angles of incidence of 30° or less.

4. The antirachitic potency of milk irradiated with ultra-violet radiation striking milk films at angles of incidence less than 90° and as low as 30° is the same as that obtained when the angle of incidence is 90°, other things being equal.

REFERENCES

- (1) SUPPLEE, G. C., BECK, H. H., AND DORCAS, M. J. Irradiated milk: The influence of the intensity and character of the radiations on the antirachitic potency. *Jour. Biol. Chem.* **98**: 769. 1932.
- (2) SUPPLEE, G. C., AND DORCAS, M. J. Irradiated milk: The transmission and antirachitic activation of milk films by ultra-violet radiations. *JOUR. DAIRY SCI.* **17**: 433. 1934.
- (3) SUPPLEE, G. C., FLANIGAN, G. E., BENDER, R. C., AND DORCAS, M. J. Irradiated milk: The influence of fat content and time of exposure on the antirachitic potency. *JOUR. DAIRY SCI.* **17**: 483. 1934.
- (4) SUPPLEE, G. C., AND DORCAS, M. J. Irradiated milk: Characteristics of the flowing film required for optimum efficiency of antirachitic activation. *JOUR. DAIRY SCI.* **17**: 524. 1934.
- (5) SUPPLEE, G. C., HANFORD, Z. M., DORCAS, M. J., AND BECK, H. H. Irradiated milk: The amount of vitamin D and its rate of formation. *Jour. Biol. Chem.* **95**: 687. 1932.

NUMBERS OF MICROORGANISMS FALLING FROM THE AIR IN DAIRY PLANTS*

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The air has long been recognized as one of the sources of the microorganisms in dairy products. The data reported in the literature (*e.g.*, 9) indicate that the numbers of organisms falling into milk from the air in stables are relatively small, especially when the air is reasonably free from dust, and it seems probable that in dairy plants comparatively few organisms fall into the various products from the air. However, under certain conditions, a small number of organisms may be of great importance. The organisms may fall into a product in which they can grow or they may fall on equipment where moisture and nutrients permit growth so that the contamination from this equipment is relatively heavy.

It is sometimes assumed that the microorganisms in the air of a dairy plant originate largely in the plant itself and in some cases attempts are made to reduce the numbers of organisms in the air by the treatment of certain rooms in a plant. For example, some butter printing rooms are fumigated in order to reduce the danger of the butter developing mold growth. Commonly, such treated rooms are thrown open to the outside air so that the comparative numbers of microorganisms in the air inside and outside a plant become of importance.

STATEMENT OF PROBLEM

The work herein reported was undertaken to determine (a) the numbers of bacteria, yeasts and molds falling from the air in rooms in which dairy products were being handled and also from the outside air; (b) the influence of season on the numbers of microorganisms falling from the air and (c) the protective effect of a muslin covering on the numbers of microorganisms falling on a surface.

GENERAL PROCEDURE

Once each week, for approximately a year, petri plates containing agar were exposed to the air in three dairy laboratories and in an experiment station bacteriological laboratory in regular use at the Iowa State College, and also to the outside air. Each period of exposure was from 10:30 to 11:30 A.M. The plates were regularly protected from flies, etc., by a wire screen (12 meshes to the inch) mounted on a supporting frame that held

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the screen about 1 inch from the surface of the agar; the screen and frame were regularly sterilized just before use. During a part of the year additional plates were exposed at some of the stations under pieces of sterilized muslin that were held over the agar by folding under the plates and holding the edges together with gummed labels. After the exposure, the covers of the plates were replaced, the plates incubated and the colonies developing then counted.

There are certain objections to the method used that should be recognized in interpreting the results. Some of these are as follows: (a) the surface exposed is relatively small and may not be exactly representative of the conditions being studied; (b) some of the microorganisms falling on the agar may not encounter conditions satisfactory for growth; (c) some of the microorganisms falling may be held away from the agar by particles of dust and thus fail to develop; (d) some of the colonies developing may represent a group or clump rather than a single organism; (e) the edge of a plate may form a shelter which favors the settling of particles of dust, etc. In general, the results secured under comparable conditions were uniform.

REVIEW OF LITERATURE

Frankland and Hart (4), at London, England, determined the numbers of microorganisms in 10 liters of air with an aeroscope and also the numbers falling from the air by exposing gelatin plates; the results of the latter trials were expressed as the numbers of organisms falling per square foot per minute. The determinations were made at various intervals over a period of approximately a year and involved the air within buildings as well as the outside air. The bacteria, yeasts and molds were not counted separately. The general results showed that throughout the year considerable numbers of microorganisms were present in the air and were falling upon the exposed surfaces, both indoors and outside. In general, the numbers of organisms in the air during the summer months were considerably greater than during the winter months.

The numbers of microorganisms in the air at Winnipeg, Canada, were studied by Buller and Lowe (2); they (a) filtered a definite volume of air (usually 10 liters) through glass wool and finely powdered sugar and (b) exposed gelatin plates to the air for varying periods (usually 1 to 15 minutes). The results of the former method were expressed as the numbers of microorganisms per 10 liters of air, and of the latter method as the numbers falling per square foot per minute. Bacteria, yeasts and molds were not counted separately. With both methods the smallest numbers of microorganisms were found during the winter and the largest numbers during the summer. The average number falling per square foot per minute was 19.5 for the period from the first week in November to the first week in

April, while for the rest of the year it was 1206.6. The highest monthly averages, which were for October and April, coincided with the windiest periods of the year and the counts obtained on windy days were usually very high. Commonly, bacteria were the predominant organisms developing on the plates, although molds were frequently numerous.

Fabian (3) determined the numbers of bacteria and molds in the air of an ice cream plant at irregular intervals, over a period of approximately a year, in two ways: (a) by the use of an aeroscope and (b) by exposing 10 ml. portions of sterile saline solution, contained in deep culture plates, to the air for five minutes and plating. He found that the numbers of bacteria in the air of the ice cream plant were insignificant in the contamination of ice cream, although his results showed that considerable numbers of bacteria and molds were present in the air and were falling on the exposed plates. The numbers varied greatly and there were considerably more bacteria than molds. The largest numbers of bacteria were found during April and the smallest during January. Fabian concluded that the weather was the most important single factor in determining the numbers of microorganisms in the air. The majority of the bacterial types present, as determined on milk powder agar, were peptonizers, alkali formers and inert forms, with few acid producers.

The air in a butter plant was considered by Grimes, Kennelly and Cummins (5) as a possible source of mold contamination of butter. They emphasized the danger of air contamination of cream subsequent to pasteurization and of butter during the packing process.

Macy, Coulter and Combs (6) reported results obtained by exposing acidulated whey agar plates for 10 minutes to the air (a) over a churn, (b) over vats and (c) in a refrigerator. The exposures were made each month from July to April. Their results indicated that considerable numbers of yeasts and molds were falling from the air in the creamery studied, particularly during the summer months.

Olson and Hammer (7) exposed malt agar plates (pH 3.5) and beef infusion agar plates inside churns and also near a churn at frequent intervals during a period of several months. The results showed that considerable numbers of bacteria, yeasts and molds were falling from the air inside the churns and that larger numbers were falling near a churn. Commonly, the numbers of bacteria falling were larger than the numbers of yeasts or molds, and the numbers of molds were larger than the numbers of yeasts. In a few comparisons in which one set of plates was exposed in a churn protected with a muslin door covering while another set was exposed in an unprotected churn, the numbers of organisms falling in the protected churn were considerably less than the numbers falling in the unprotected churn; the decrease was especially striking with the bacteria.

DETAILED METHODS

The numbers of bacteria falling from the air were determined with beef infusion agar while the numbers of yeasts and molds were determined with malt agar (made from the Difco dehydrated product), adjusted to pH 3.5 with lactic acid. After exposure, the plates were ordinarily incubated at about 21° C. for four days and the colonies then counted with the aid of a low power binocular, or estimated if the numbers were very high; occasionally, it was necessary to count the malt agar plates after incubating two or three days because of a heavy development of molds. In counting the malt agar plates, the yeasts and molds were counted separately. The bacterial counts undoubtedly included the yeasts also because detailed examinations of the individual colonies were not made; however, the numbers of yeasts were unusually small compared to the numbers of bacteria.

The results were expressed as the numbers of organisms falling on a 90 mm. (diameter of the bottom) petri plate per hour. The breaks in the data are due to failures to make the exposures or to freezing temperatures or rainfall spoiling the plates.

The six locations at which exposures were made are as follows: (a) outside east, a stone ledge about two feet high on the roof of the dairy industry building; (b) outside west, a similar ledge about 100 feet away from the first; (c) butter laboratory, on top of a switch box about five feet from the floor near the center of the room; (d) market milk laboratory, on top of a switch box about five feet from the floor near the center of the room; (e) cheese laboratory, on top of a switch box about five feet from the floor near the wall; and (f) experiment station laboratory, on a shelf about two feet above a laboratory desk. Muslin protected plates were exposed only at locations (b) (c) and (f).

RESULTS OBTAINED

The data obtained on the numbers of bacteria, yeasts and molds falling from the air are summarized in table 1. The general results indicate that microorganisms were regularly falling from the air and that, among these organisms, bacteria were the most numerous and yeasts the least numerous; with each of the groups of organisms the numbers were variable.

The detailed data, from which the summary was prepared, show the following relationships. With the 240 bacterial and the 290 mold counts there were no instances in which the counts were 0 while with the 290 yeast counts there were 69 instances in which the counts were 0, the greatest number of these occurring with the counts obtained in the experiment station laboratory. In the 240 comparisons of the bacterial and mold counts, the bacterial counts were the higher in 174 instances and the mold counts in 66; 42 of the 66 instances occurred during the last two months of the study

TABLE 1
Microorganisms falling from the air. Numbers per 90 mm. petri plate per hour

LOCATION	NO. OF TRIALS	BACTERIA		
		Organisms per plate		
		min.	max.	av.
Outside east	38	6	2024	161.8
Outside west	38	12	2034	167.7
Butter lab.	41	18	516	166.5
Market milk lab.	41	7	734	185.9
Cheese lab.	41	12	686	144.7
Expt. sta. lab.	41	2	179	39.4

YEASTS				
Outside east	47	0	83	11.7
Outside west	47	0	85	12.1
Butter lab.	49	0	21	4.6
Market milk lab.	49	0	25	4.5
Cheese lab.	49	0	43	4.4
Expt. sta. lab.	49	0	16	2.0

MOLDS				
Outside east	47	4	275	74.9
Outside west	47	2	282	67.6
Butter lab.	49	2	302	60.3
Market milk lab.	49	3	256	55.1
Cheese lab.	49	2	286	48.3
Expt. sta. lab.	49	1	666	79.9

when the mold counts were especially high. There were more instances in which the mold counts were higher than the bacterial counts with the exposures made in the experiment station laboratory than with those made in any of the other five locations; in this location the bacterial counts were often comparatively low while the mold counts were often comparatively high. In the 290 comparison of the mold and yeast counts, the mold counts were the higher in 275 instances and the yeast counts in 9, while in 6 there was no difference.

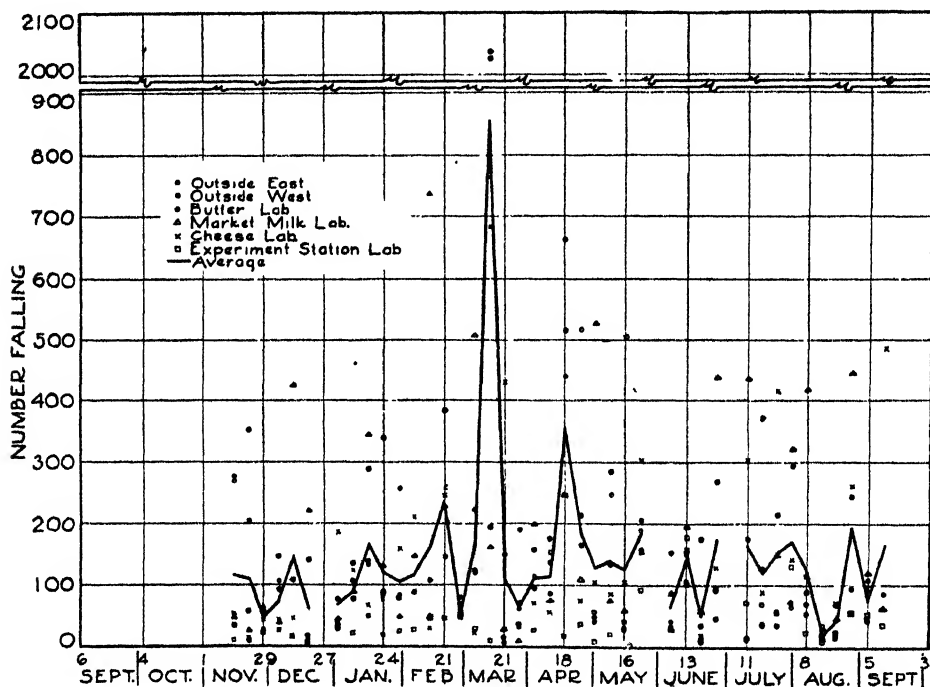
A comparison of the average numbers of bacteria, yeasts and molds falling at the outside locations with the numbers falling at the locations indoors shows:

1. Except for the comparatively low value for the experiment station laboratory, the average numbers of bacteria falling indoors did not differ significantly from those falling outside. However, the maximum value for each of the outside locations was considerably higher than the maximum value for each of the locations indoors.

2. The average numbers of yeasts falling indoors were smaller than the numbers falling outside and the maximum value for each of the outside locations was higher than the maximum value for each of the locations indoors.

3. The average numbers of molds falling indoors did not differ significantly from the numbers falling outside.

A study of the data from which the averages were prepared shows that, with a group of exposures made at the same time, it was much more common to secure higher counts outside than indoors with the yeasts than with the bacteria or the molds.

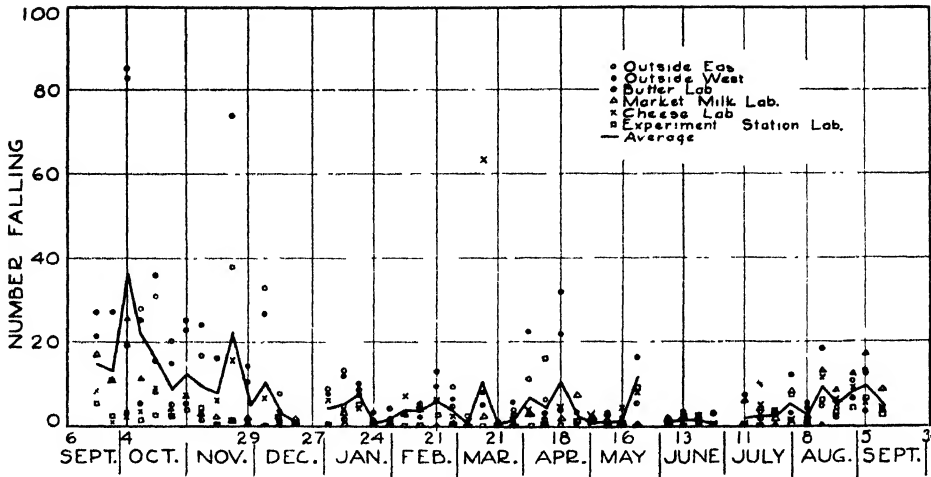


GRAPH 1.

Bacteria falling from the air. Number per 90 mm. petri plate per hour.

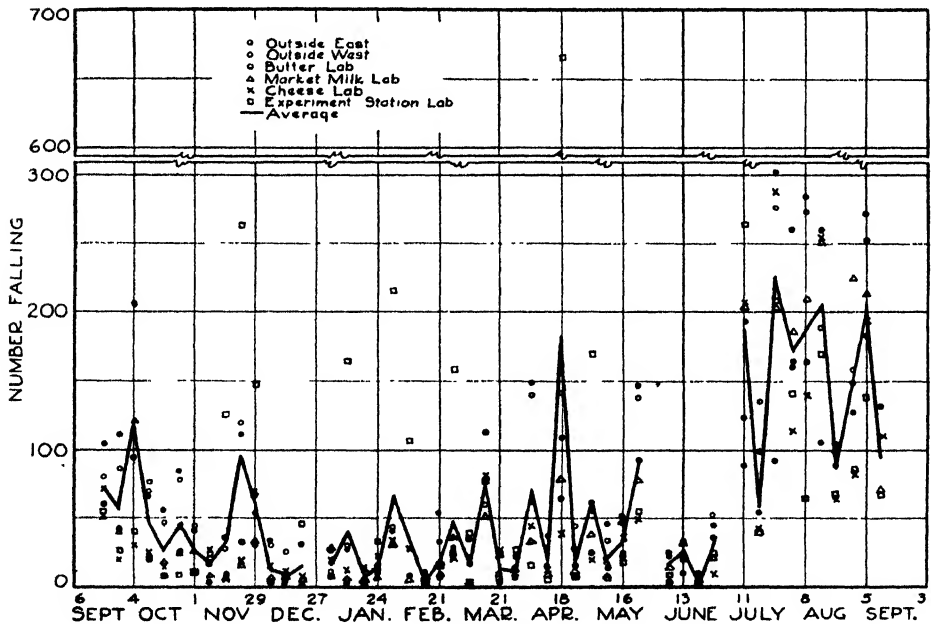
In general, the results indicate that the numbers of bacteria, yeasts and molds falling from the air in dairy laboratories do not differ greatly from the numbers falling from the outside air.

The average numbers of bacteria, yeasts and molds falling from the air at the various examinations, together with the numbers falling at the different locations, are shown in graphs 1, 2 and 3. There were no well defined seasonal trends in the numbers of the various microorganisms falling. The bacterial counts tended to be the highest in March and April, the yeast counts in October and November and the mold counts in July, August and



GRAPH 2.

Yeasts falling from the air. Number per 90 mm. petri plate per hour.



GRAPH 3.

Molds falling from the air. Number per 90 mm. petri plate per hour.

September, but there were always great variations between the counts obtained on dates that were close together.

In an attempt to correlate the variations in counts with the climatic conditions, the official weather bureau records of the daily precipitation during

the period of the investigation and of the daily wind movement and evaporation from April 3, 1933, to September 12, 1933, were studied; these were supplemented with notes taken on the days of exposure. There was no close correlation between any of the climatic factors and the counts obtained either outside or indoors, but there are several observations of interest. The counts obtained outside were usually rather low when rain had fallen during the 24 hours preceding the exposures, due presumably to the partial removal of organisms from the air by rain. The highest bacterial counts outside were obtained on a very windy day in March but there was no close correlation between wind velocity and the counts outside. The counts ob-

TABLE 2

Effect of a muslin covering on the microorganisms falling from the air. Numbers per 90 mm petri plate per hour

LOCATION	PROTECTION	NO. OF TRIALS	BACTERIA		
			Organisms per plate		
			min	max	av
Outside west	Unprotected	34	12	2034	182.3
	Protected with muslin	34	0	990	53.0
Butter lab	Unprotected	37	18	516	178.2
	Protected with muslin	37	0	56	10.0
Expt. sta. lab	Unprotected	37	2	179	42.0
	Protected with muslin	37	0	82	5.0
YEASTS					
Outside west	Unprotected	35	0	35	5.9
	Protected with muslin	35	0	8	1.2
Butter lab	Unprotected	37	0	18	4.0
	Protected with muslin	37	0	2	0.2
Expt. sta. lab	Unprotected	37	0	16	2.0
	Protected with muslin	37	0	2	0.1
MOLDS					
Outside west	Unprotected	35	2	282	66.6
	Protected with muslin	35	0	114	13.6
Butter lab.	Unprotected	37	2	302	69.8
	Protected with muslin	37	0	20	5.8
Expt. sta. lab	Unprotected	37	1	666	84.5
	Protected with muslin	37	0	36	5.2

tained outside during the long hot, dry period from May 29 to June 25, 1933, were rather low; this may have been due to the destruction of organisms by dessication during this period. The mold counts were especially high soon after a rain on June 25 and continued to be high during the ensuing weeks of warm weather and frequent precipitation; the bacterial and yeast counts, however, did not show any conspicuous tendency to increase during this period.

The data dealing with the effect of a muslin covering on the numbers of bacteria, yeasts and molds falling on an agar surface are summarized in table 2. The average numbers of bacteria, yeasts or molds falling at any location were regularly much smaller with the muslin protection than without it. The protective action of the muslin is convincingly shown in the detailed data from which the summary was prepared. The numbers of

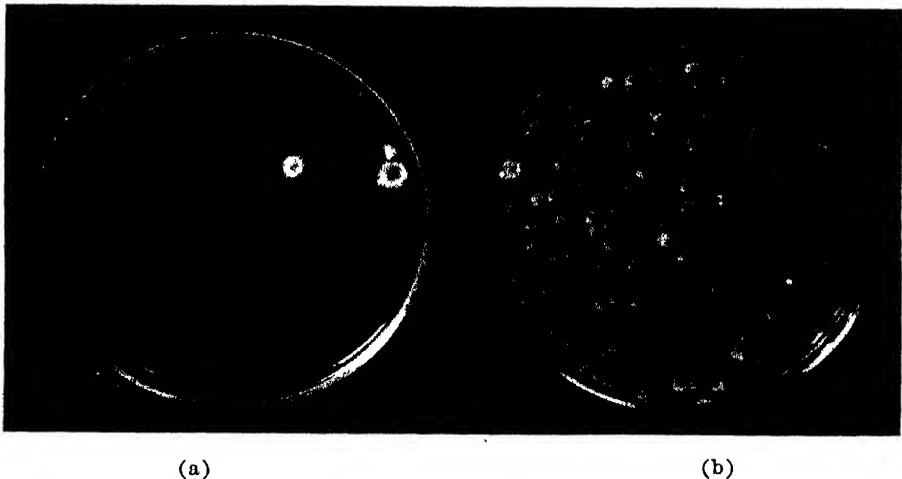


FIG. 1. PROTECTIVE ACTION OF MUSLIN AGAINST MOLDS

(a) Plate protected with muslin. (b) Plate unprotected except for coarse screen. Plates exposed one hour in experiment station laboratory.

yeasts falling with or without the muslin protection were frequently too small for the difference to be of significance but in the 109 comparisons the numbers were smaller with the protection in 69 instances, larger in 5, equal in 3, and, in 32, yeasts were not found either with or without the muslin covering. The numbers of bacteria and molds falling in the comparisons (108 for the bacteria and 109 for the molds) were regularly smaller with the muslin protection than without it. Figure 1 illustrates the protective action of the muslin against molds; the plates were exposed in the experiment station laboratory. There were always smaller variations in the counts secured at the different stations when the plates were protected with muslin than when they were not.

From the average numbers of bacteria, yeasts or molds falling at the various locations with and without protection, it appears that the muslin gave less protection outside than it did indoors. This may have been due to the greater air movement outside.

DISCUSSION OF RESULTS

Since the results obtained indicate that bacteria, yeasts and molds were constantly falling from the air in the various dairy laboratories, it is evident that the contamination of dairy products and plant equipment from the air is to be expected. While the numbers of organisms coming from the air is not large when the products or equipment are given reasonable protection, small numbers of organisms may lead to disastrous results under certain conditions. One mold spore falling on a piece of butter may develop and thus be very serious, both from the standpoint of the appearance and the flavor and odor of the product. If the mold spore falls into pasteurized cream or on equipment and is eventually carried to a piece of butter at a point where it can grow, the result may also be serious.

The contamination of equipment from the air may be considerable if it is allowed to stand unprotected for extended periods. This is indicated by the work of Olson and Hammer (8) who prepared agar discs from metal surfaces before and after exposure to the air; molds were especially conspicuous on the discs prepared after exposure.

With as many or more organisms falling from the outside air as from the air in dairy plants, the air coming into plants through windows and doors should be considered a source of microorganisms along with the air that has been in the plant for some time. Areas of mold growth are rather frequently encountered on walls, ceilings, moist wood, etc., in dairy plants and these undoubtedly contribute mold spores to the air of the plants but mold spores may also be carried into plants from outside sources. In this connection the work of Bisby, Jamison and Timonin (1) is important since it shows that the molds isolated from butter were largely soil forms. Approximately half of the 65 identified fungi from Manitoba butter were also isolated from the soil there. These investigators state, "It is probable that the fungi recorded as found in butter nearly all arise from spores or bits of mycelium from the soil, plants, débris, and manure; they are carried by the air or dust particles and contaminate the cream, equipment or butter. Most of the fungi found in butter produce spores abundantly." While colonies of bacteria and yeasts are less commonly seen about a dairy plant than mold colonies, yeasts and bacteria can grow in milk, cream, etc., and some of them be carried into the air in one way or another. These organisms can also evidently be carried into dairy plants by outside air.

Any attempt to control air contamination in a plant should include a consideration of the outside air coming into the plant. This may be impor-

tant in connection with the printing of butter, especially unsalted butter, and with certain other products.

The protection from the air that is secured by a muslin covering, while by no means complete, may be of importance under certain conditions. Olson and Hammer (7) found fewer organisms falling in a churn having a muslin cover over the door than in a churn with the door unprotected. When such materials as cream must be held for extended periods in vats that have no covers some protection can be secured with muslin. Even incomplete protection may be distinctly useful with pasteurized cream intended for unsalted or low salted butter and with comparable materials.

CONCLUSIONS

Bacteria, yeasts and molds were rather constantly falling on agar exposed at the six locations studied, four of which were indoors and two outside; the four locations indoors included three dairy laboratories and an experiment station laboratory. In general, bacteria were the most numerous and yeasts the least numerous.

The numbers of microorganisms falling from the air indoors did not differ greatly from the numbers falling from the outside air.

There was no distinct seasonal variation in the numbers of bacteria, yeasts or molds falling from the air at the various locations studied.

A muslin cover reduced the number of organisms falling on a surface from the air.

REFERENCES

- (1) BISBY, G. R., JAMIESON, M. C., AND TIMONIN, M. The fungi found in butter. *Can. Jour. Res.* 9: 97-107. 1933.
- (2) BULLER, A. H. R., AND LOWE, C. W. Upon the number of microorganisms in the air of Winnipeg. *Proc. Trans. Roy. Soc. Can.* 4 (Sec. 4): 41-58. 1911.
- (3) FABIAN, F. W. A study of the sanitary significance of air in relation to ice cream. *Mich. Agr. Exp. Sta. Tech. Bul.* 83. 1927.
- (4) FRANKLAND, P. F., AND HART, T. G. Further experiments on the distribution of micro-organisms in air (by Hesse's method). *Proc. Roy. Soc. London* 42: 267-282. 1887.
- (5) GRIMES, M., KENNELLY, V. C. E., AND CUMMINS, H. A. A study of fungi found in butter. *Sci. Proc. Roy. Dublin Soc., n.s.*, 19: 549-569. 1930.
- (6) MACY, H., COULTER, S. T., AND COMBS, W. B. Observations on the quantitative changes in the microflora during the manufacture and storage of butter. *Minn. Agr. Exp. Sta. Tech. Bul.* 62: 12. 1932.
- (7) OLSON, H. C., AND HAMMER, B. W. Bacteriology of butter. V. Studies on the microorganisms in churns. *Ia. Agr. Exp. Sta. Res. Bul.* 159: 94-96. 1933.
- (8) OLSON, H. C., AND HAMMER, B. W. The agar disc method for studying the contamination from metal surfaces. *Ia. Agr. Exp. Sta. Bul.* 300. 1933.
- (9) RUEHLE, G. L. A., AND KULP, W. L. Germ content of stable air and its effect upon the germ content of milk. *N. Y. (Geneva) Agr. Exp. Sta. Bul.* 409. 1915.

FRESHENING AGES OF PUREBRED COWS IN IOWA COW TESTING ASSOCIATIONS*

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The age at which dairy heifers should be bred for first freshening has been the object of only a few controlled experiments, although it is a subject of much practical importance, and recommendations about it are found in almost every treatise on dairy farming. Still fewer have been any surveys of dairy farming practice intended to ascertain at what ages dairy heifers actually freshen for the first time. Data collected at this Station for another purpose were found suitable for such a survey of dairy breeding practice and the results, which are surprising in some respects, are presented here.

Widely divergent views are sometimes held on the desirable age for first calving. We quote (through the courtesy of Mr. Houghton Seaverns of the Holstein-Friesian Association) two extreme views expressed by breeders who were being asked for additional information about calves out of dams less than one year old when bred. 1. From a Wisconsin breeder in 1926: "I believe in breeding heifers when they are a year old and not any older. I have kept them until they were two years old and then served them but never had any luck with them. They turned out to be big steers and nothing else." 2. From a New York breeder in 1926: "One of our best and most successful breeders here makes it a rule to breed them when they first come in heat no matter at what age." Such views as these are unusual. More widely accepted as ideals are such recommendations as those of Eckles (2), who stated on the basis of studies in the herd of the University of Missouri and at the Maryland Station that normally developed heifers should be bred to calve at the following ages: Holsteins at 28-30 months, Ayrshires at 27-29 months, Guernseys at 26-28 months and Jerseys at 24-26 months, respectively. Henry and Morrison (5) recommend that well fed Jersey and Guernsey heifers should be bred to calve at an age of 24-28 months while Holsteins, Ayrshires and Brown Swiss should not be bred to calve before 28-36 months old.

However, Reed, Fitch and Cave (7) found that even though Holstein heifers bred to calve at 24 months of age did not develop as well as animals on the same feed bred to calve at 30 months of age, yet their milk producing ability was not affected by early calving. Turner (9) found that the mean ages for cows placed on official test for the first time at ages up to and

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including 3 years were 29.8 months for Ayrshires, 28.5 months for Guernseys, 28.8 months for Holsteins and 27.3 months for Jersey cows, corresponding approximately to the ages recommended by Eckles. However some of those cows may really have freshened their second time when they were started on test before 36 months of age. Turner concludes that "... the most efficient milk and fat production (utilization of nutrients) will be obtained by breeding animals to calve at from 20 to 24 months of age, maximum production at about 30 months of age, and within 5 to 10

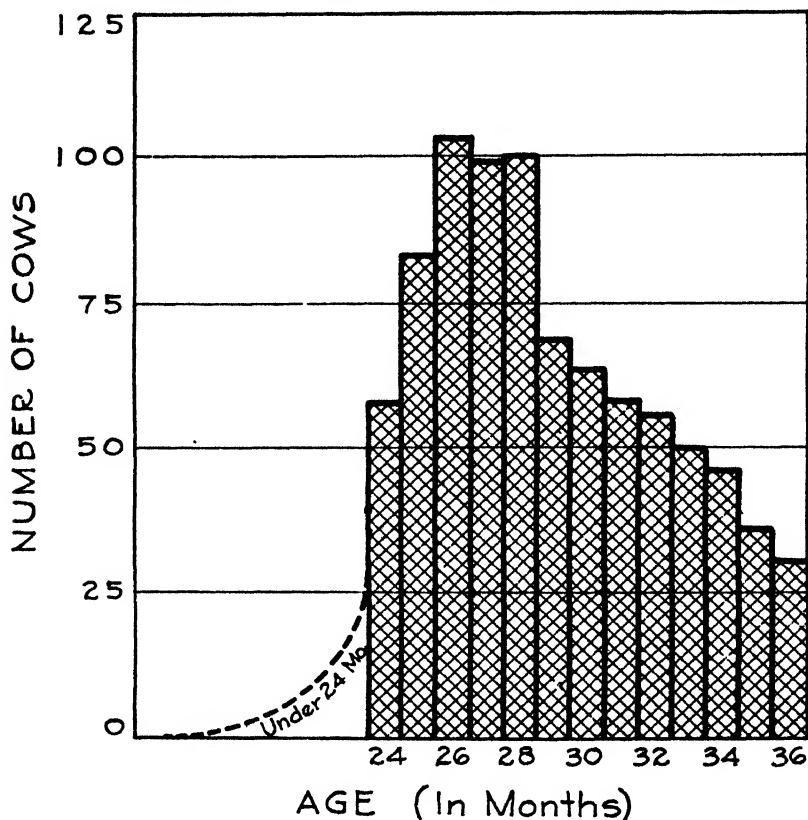


FIG. 1. Distribution of calving ages of those Holstein-Friesian cows which completed their first Official Records during 1933 after freshening at ages of 36 months or less. (Data from H. W. Norton, Jr.).

per cent of the maximum production at from 23 to 28 months depending upon the breed." Through the courtesy of Mr. H. W. Norton, Jr., we show in figure 1 the age distribution of those Holstein-Friesian cows which, freshening at 36 months or less, finished their first Official Records in 1933. This distribution is not greatly different from Turner's (Fig. 2), as is to be expected since the two groups of data are similarly collected, except for

the dates in which the records were completed. Figure 1 does seem to show slightly more skewness than Turner's data but that discrepancy may not be significant.

Aside from Turner's study, which was confined to cows on official test, we find no systematic survey of dairy breeding practices in this respect. A study by Lush and Lacy (6) of the ages of the dams of registered cattle of five dairy and three beef breeds did show that there are fewer cows calv-

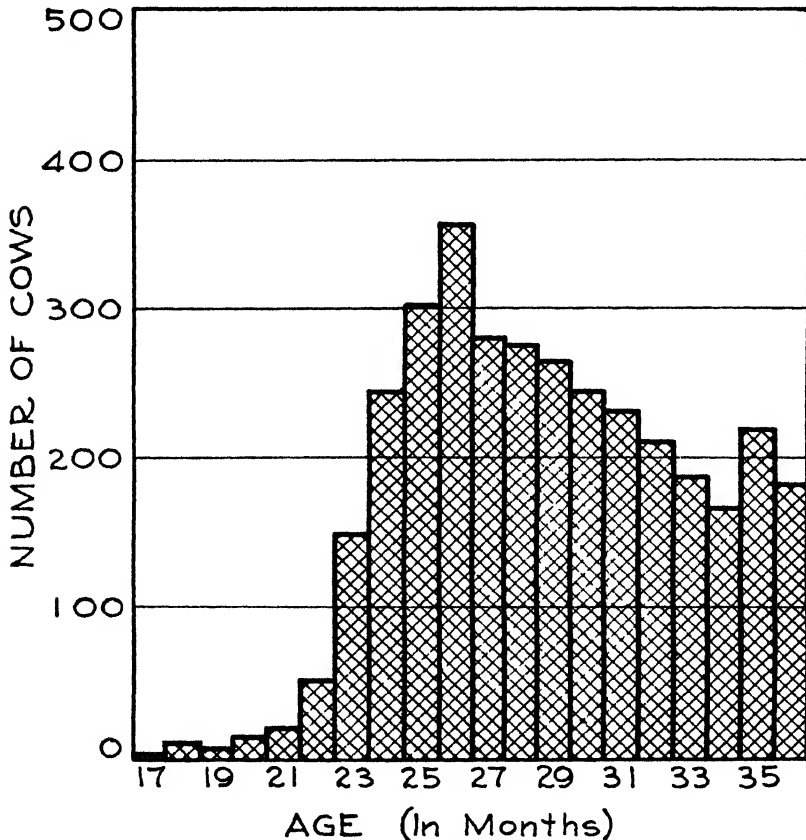


FIG. 2. Distribution of the calving ages of those Holstein-Friesian cows which have their first A.R.S.O. records reported in volumes 18 to 34 (records finished prior to April 1, 1923) and which began these records at ages of 36 months or less. (Turner's data).

ing for the first time after 36 months of age than die or are culled, so that in a population static in numbers, the maximum number of cows at any one calving age is passed some time earlier than 36 months. However their data were presented only by six-month intervals and (as in Turner's study) they did not know which calvings at ages less than 36 months really were first calvings. A study in Great Britain by Smith and Robison (8) similar to that by Lush and Lacy led to very much the same conclusions.

There have been a few authentic cases of heifers calving at less than 12 months of age but such cases throw little light on general practice because their extreme rarity makes them much more apt to be reported than are calvings at usual ages.

SOURCE OF DATA

The data for this investigation were obtained from the standard record books borrowed (with the cooperation of the Dairy Husbandry Extension Service) from owners who had been members of Iowa Cow Testing Associations for at least three consecutive years. Grade cows are not included in the present paper because the exact birth dates were rarely stated for grades. Most of the data comes from the years 1926 to 1932, although a few come from as far back as 1920. Figure 3 shows the location of the

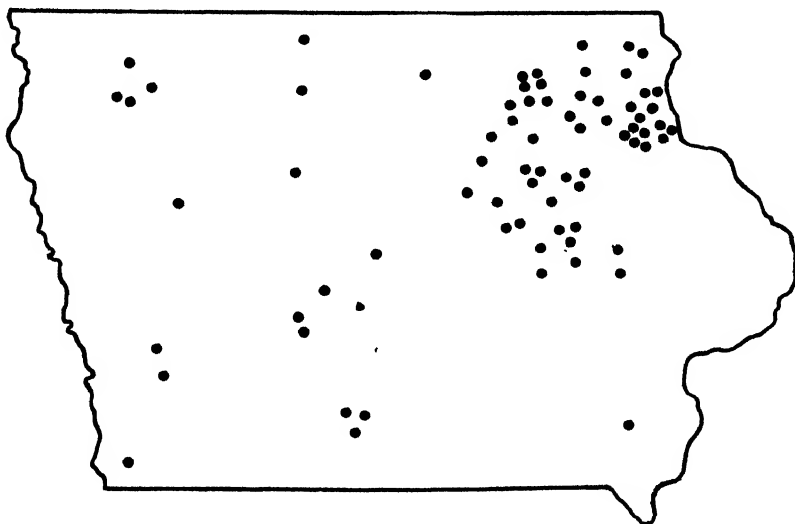


FIG. 3. The location of the herds from which these Iowa Cow Testing Association data came.

herds, most of which are in the somewhat specialized dairy section of northeastern Iowa.

For reasons connected with the primary purpose of the investigation, only those lactations which lasted at least nine months were used. Altogether there were 1073 purebred cows with a total of 2541 such lactations where the exact calving ages were known and verified from the herdbooks of the breed. Age is expressed here as the last attained number of months. For example, all cows calving at from exactly 23 months old to 23 months and 29 days are classed as 23 months old, thus making their real ages about a half month more than the following averages and graphs show.

AGE AT FIRST CALVING

In most cases when a heifer calves for the first time the record books contain a specific note that this is a first calving. In some cases (all but seven of which were among Holsteins) where this was not definitely stated, the general evidence (*i.e.*, age and other information which could be obtained from the current or the preceding record book) indicated that the calving really was a case of first calving. Such cows are included in our data as first calf heifers. That any bias is introduced by such inclusions seems unlikely from figure 4, which shows separately the ages of the 283 Holsteins

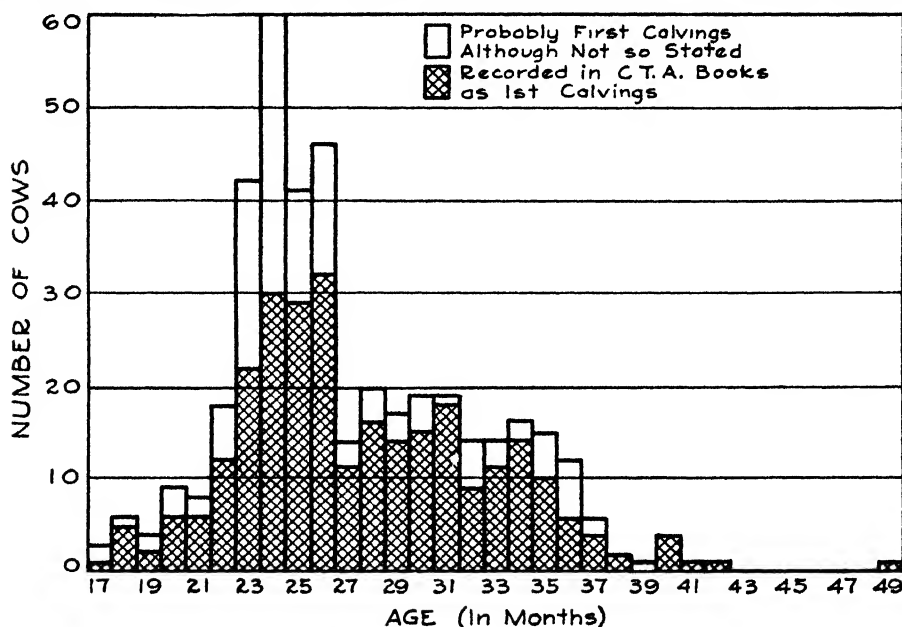


FIG. 4. Distribution of the ages of 415 registered Holstein-Friesian heifers at first calving in Iowa Cow Testing Associations.

definitely stated to be first calvers and the 132 Holsteins not so stated, but indicated by other evidence to have been first calvers. The extreme cases of first calving at ages above 40 months were all expressly stated to be first calvers.

Figure 4 shows that the distribution is extremely skew. The mode or age at which more freshen than at any other one age, is at 24 months but the mean is in the 27th month, being much affected by a few extremely late first calvers. The age before which half of all heifers freshen (the median age) is 25 months and 10 days in terms of figure 4 or about 25 months and 25 days in actual age when allowance is made for the fact that the ages in figure 4 are computed as the last attained full month. Hence a purebred cow in Iowa Cow Testing Associations is to be regarded as a later calver

than most if she is as much as 26 months old when she first freshens. The mode, median and mean are practically the same for all cows as they are for Holsteins (Fig. 4), the earlier freshening of Jerseys and Guernseys (Table 1) being almost exactly balanced by the very late first freshening

TABLE 1
Ages of heifers at first freshening, by breeds

BREED	NO. OF COWS	MEAN AGE IN MONTHS
Ayrshire	3	28.3 \pm 2.8*
Guernsey	34	25.5 \pm .8
Holstein	415	27.2 \pm .2
Jersey	95	25.5 \pm .5
Shorthorn	4	31.0 \pm 2.5
Red Polled	19	34.9 \pm 1.1
Total	570	27.1

* The figures after the \pm signs are standard errors of these means and are computed from the average intra-breed standard deviation of 4.91 months.

dates for the few Shorthorns and Red Polls included. Other studies of the breeding efficiency of dairy herds have generally shown that about half of those cows which eventually conceive do so on the first service, about half of the remainder do so on the second service, about half of the remainder conceive the third service, and so on. This in itself, if true also in Iowa Cow Testing Association herds, would give some such skewness as that in Figure 4 even if most breeders intended to have freshening occur exactly at 24 months. On account of this lack of perfect control over breeding, the average age at first calving will usually lag behind what the dairymen think is ideal.

BREED AND AGE AT FIRST CALVING

The number of cows of the different breeds and their average calving age is given in table 1. The mean age at first calving varies from about 25.5 months for Jersey and Guernsey cows to almost 35 months for Red Polled cows. The analysis of variance (Table 2) shows that there are statistically quite significant (Fisher, 3) breed differences in this group of data. However those differences are not a very large part of the total causes of variation in age at first calving in this population, since the variance within breeds (24) is nearly 90 per cent as large as the variance (27) in the whole population considered as a unit. When the individual breed averages (Table 1) are compared, it is seen that the Red Polled cattle (all of which came from one herd) are significantly older at first calving than any of the other breeds, except perhaps the Shorthorns and Ayrshires, whose numbers are too small to warrant much confidence in any estimate of statistical significance. The Holsteins are significantly older than the Jerseys

TABLE 2

Analysis of variance in first calving age for cows of different breeds, from different herds, calving at different months and fed differently

SOURCE OF VARIATION	D/F	MEAN SQUARE	σ	P
Between breeds . . .	5	311		far less than .01
Within breeds	564	24	4.91	
Between herds within breeds	60	55		about .03
Within herds . . .	504	20	4.52	
Between months . . .	11	53		far less than .01
Within months	558	26	5.11	
Total . . .	569	27	5.16	
Between feed grade groups	6	51		about .06 or .07
Within feed grade groups	562	26	5.14	
Total . . .	568*	27	5.16	

* Unintentionally the feeding of one cow was not graded.

(of which 60 out of the 95 were from one herd). Probably the difference between Holsteins and Guernseys is significant, although the small numbers of the latter still leave that open to some doubt. The lowness of the Jersey average age cannot be attributed to any peculiar management of the one herd from which most of the Jersey first calvings come, because the average age of the 60 first calvings in that herd was 25.8 months as compared with 25.5 months for the entire 95 Jersey first calvings. However in the case of the Red Polls we cannot know whether this is really a breed difference or a difference in the herd manager's policy, since all the data on this point came from one herd.

TIME OF YEAR AND AGE AT FIRST CALVING

First calvings, when classified according to the month of calving, might have a mean age which is significantly different from month to month if dairymen make much effort to hold back some heifers and to breed others earlier in order to have most of them calve at a certain season.

The mean age at which the heifers freshen in the different months is given in table 3. The analysis of variance in table 2 shows that the month effect is probably significant (P is about .03) but not very important. The standard deviation within months (5.11) is but slightly smaller than that (5.16) in the whole population. Examination of the individual monthly averages does indicate some slight tendency to postpone the breeding for those which would otherwise calve in the late spring or early summer so that they calve a little older than usual in order to freshen in August or September. The August and September ages at first calving are signifi-

TABLE 3
Number and proportion of first calvings by months

MONTH OF FRESHENING	NO. OF COWS	MEAN AGE IN MONTHS*	% FRESHENING IN EACH MONTH	
			These data	Beard's data
January	34	27.1	6.0	9.1
February	32	27.3	5.6	8.6
March	35	26.3	6.1	7.7
April	43	26.1	7.5	6.5
May	21	25.0	3.7	3.3
June	23	25.7	4.0	3.6
July	21	25.6	3.7	4.5
August	38	28.0	6.7	8.8
September	74	29.0	13.0	14.2
October	101	27.3	17.7	11.3
November	81	27.2	14.2	13.4
December	67	26.6	11.8	9.0
Total	570	27.1	100	100

* The standard errors of these means range from .49 for the months with only 21 calvings to .22 for October with 101 calvings.

cantly or almost significantly older than those in the three to five immediately preceding months. Also included in table 3 is the seasonal distribution (from an unpublished study by Beard, (1)) of the calvings of the 815 cows reported as "two" years old in Cow Testing Association herds in Iowa during 1930-31. The only important discrepancies between Beard's data and ours are for October and for January and February. Beard's data included both grades and purebreds and were from a single year. The discrepancies might be connected with either of these circumstances.

If there is a seasonal sexual cycle in cattle, it would lead to an excess of calvings during late winter and early spring months, so far as one can infer from what happens on ranches where bulls are left with the cow herd all year. Table 3 shows quite a different picture, 45 per cent of these first calvings coming in the autumn quarter of the year and nearly 57 per cent in the four months from September 1 to December 31.

MANAGEMENT AND ENVIRONMENT OF HERDS

The 570 first-calf cows came from 66 different herds, each contributing from 1 to 60 cows. The herds with 10 or more first-calf cows are shown in table 4. All herds whether large or small are included in the analysis of variance in table 2. The analysis of variance shows that the mean age at calving varies from herd to herd in a more extreme way than can be attributed to chance alone. Whether these herd differences result from avowed differences in opinions of the herd managers about the proper age

TABLE 4
Average age at first freshening in the larger herds

BREED	NUMBER OF COWS	MEAN CALVING AGE*
Holstein	10	23.7
"	14	24.1
"	13	24.3
"	20	24.4
"	11	25.3
"	18	25.6
"	11	26.3
"	15	26.7
"	11	26.9
"	31	27.3
"	52	27.8
"	12	28.2
"	16	30.1
"	17	30.1
"	30	30.4
"	25	33.0
Jersey	60	25.8
Red Polled	19	34.9

* The standard errors of these means range from .67 for the 10-cow herd, through .39 for the 30-cow herd, to .27 for the 60-cow herd.

at which to breed heifers for first calving or are secondary consequences of some other feature of the herd management or of some environmental circumstance such as climate or character of feed, these data of course do not tell. The difference is large enough to be of some importance. The standard deviation within herds (4.52) is distinctly less than that (4.91) within breeds in the whole population. The intensity of this relation corresponds roughly to an intra-class correlation of +.15 between ages at first calving for cows in the same herd.

It was thought that there might be some relation between the breeding policy and the feeding policy from herd to herd. When the original data were obtained the feeding during each lactation was scored on the basis of the feed records in the record books. An arbitrary scale running from 1 to 9 was used. Grade 1 feeding consisted of legume hay, silage, 3 kinds of grain, including wheat bran, a protein supplement, grain when on pasture and grain for dry cows. Grade 9 consisted of nonlegume hay and only one kind of whole grain. We believe that the grades represent fairly well the major differences between good and poor rations, so far as those can be judged from the record books. All this grading of the feeding was done by Mr. G. G. Gibson of the Dairy Husbandry Extension Service.

The average ages at first calving for heifers thus classified according to the way they were fed in their first lactation, are shown in table 5 and the

TABLE 5
Age at first calving for cows grouped according to feeding grade

FEED GRADE	NUMBER OF COWS	MEAN AGE*
1	70	28.8
2	161	27.1
3	82	26.3
4	102	26.7
5	101	26.7
6	44	27.4
7	9	28.8
Total	569	27.1

* The standard errors of these means are $\frac{5.1}{\sqrt{n}}$ which equals .61 for grade 1 and .40 for grade 2.

analysis of variance is shown in table 2. There seems to be some tendency for late calving in both the best fed groups and the worst fed groups, although the statistical significance (P is about .06 or .07) of this is not beyond question. Whether statistically significant or not, this relation certainly is not close enough to be important since the standard deviation (5.14) within groups receiving the same feeding grade is but very slightly smaller than that (5.16) in the whole population. The difference between the average age of those in Grade 1 and those in the next four grades comes close to statistical significance. Grade 1 feeding is rather closely comparable to Advanced Registry conditions. Perhaps it is not a mere coincidence that the cows in this group have a mean age at first calving corresponding almost exactly to the one found by Turner (9) for Advanced Registry Holstein cows.

AGE AT LATER CALVINGS

Among the first calf heifers there was a very pronounced peak in calving age at 24 months of age, over 45 per cent of all first calvings occurring at ages of 23 to 26 months, inclusive. When all the ages at calving (later calvings along with the first calvings) are assembled in one distribution (Figure 5) there is a conspicuous repetition of this peak each twelve months until at very high ages where the numbers are small this cycle becomes somewhat less distinct.

Figure 5 seems to show a deliberate attempt on the part of most dairy farmers to have their cows calve at exactly two, three, four, five, etc., years of age, rather than at intervening ages. To us the most plausible single

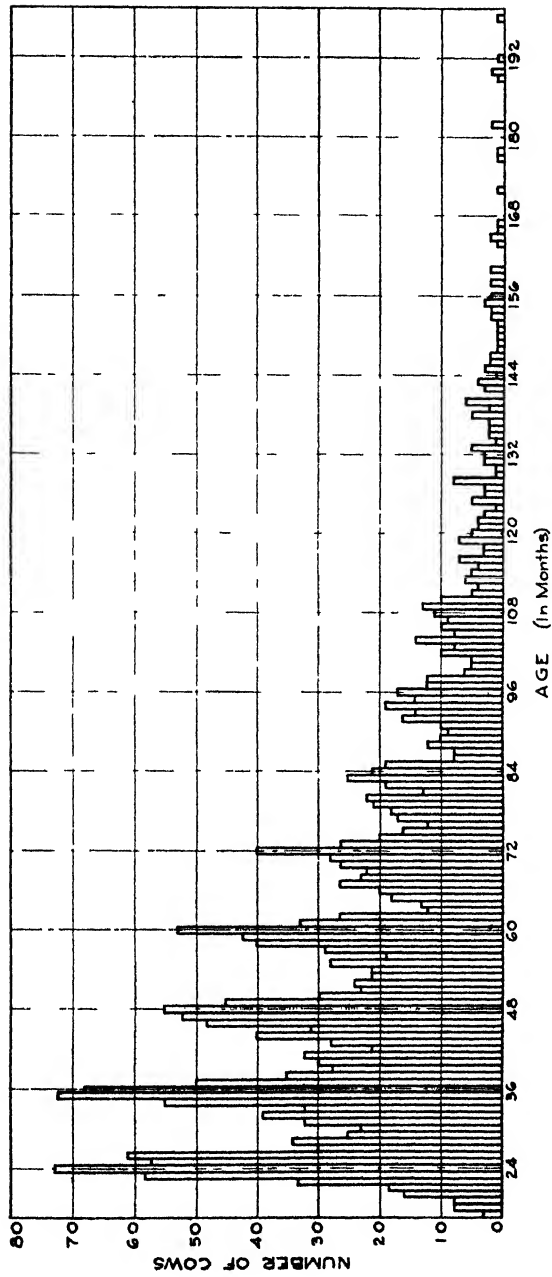


FIG. 5. Frequency distribution of the calving ages of purebred cows in Iowa Cow Testing Associations. No distinction is made here between first calvings and later calvings. There are included 1,073 different cows and a total of 2,541 calvings.

explanation seems to be that the general policy of trying to breed for fall calving would lead to a large proportion of heifers being bred to freshen in the fall. A large proportion of these same heifers would have been born in fall months and would therefore be about two years or about three years old. We are not convinced that the percentage of fall calvings in these data (Table 3) is high enough to be a complete explanation for the cycles of figure 5.

However it seems to us worth emphasizing in this connection that one cannot have a very high percentage of his cows freshen in the fall each year without also breeding his heifers to come fresh either near 24 months old or near 36 months old. Another explanation might be that most dairy farmers try rather definitely to have their heifers calve at about 24 or 25 months old and that the interval between successive calvings is so near 12 months and varies so little that the initial peak of calvings thus established at about 24 months tends to recur every 12 months, being slightly smoothed out at the higher ages by the accumulations of variations in the length of the calving intervals. We are skeptical of calving intervals being regular enough to make this explanation a major one, but it may be a contributing cause for the initially sharp cycle in figure 5 which tends to be damped down later.

Gaines (4) in studying 4,109 annual records of 740 different cows of the Red Danish breed found a similar "orderly irregularity" of the frequency distribution with the peaks at age classes 2-2.25 years and every year thereafter. The Danish records used by Gaines were all yearly records beginning at October 1st and he interprets the regularity of the peaks as being due to a preference for fall calving.

The irregularity of the calving ages with definite peaks at regular 12 month intervals is of importance in correcting for the effect of age on milk production. In finding age correction factors, a sufficiently fine grouping must be maintained as otherwise the unequal frequencies in succeeding months will tend to give the age classes with relative high frequencies less weight than they should have. In finding a regression curve it is important that the independent variable be correctly stated. Random errors in the dependent variable tend to cancel each other but that is not the case even with random errors in the independent variable. For most cows (especially for grades) the ages in Cow Testing Association records are usually reported only as a whole number of years. Figure 5 shows that there is some error in assuming that the ages are evenly distributed through each year with the mean being at or very near the half year. Moreover there was (at least in our material) a very evident tendency to state the year to the nearest year rather than to the last attained year. For example the rather large number found by a check of birth dates and calving dates to have been from 20 to 23 months old at first calving were, with few excep-

tions, entered in the record books as "two" years old and very many of those calving at ages of 31 to 35 months were listed as "three" years old. On the basis of this experience we would estimate that the average age of all cows listed as "two" years old in Cow Testing Associations records is probably about 26 or 27 months instead of the 30 months which might be expected if calving ages were evenly distributed through the year and if the ages of cows (like those of humans) were nearly always stated as of the last attained birthday.

This is a point important to keep in mind when applying to data where the age is only stated in whole years, age-correction factors derived from data where the age was stated in months or quarter years.

Figure 5 also shows vividly the youthfulness of the dairy cow population. One fourth of all purebred calves are out of dams less than 36 months old and one half are out of dams less than four years and four months old, while only one fourth are from dams as much as six years and three months old when these calves are born. To be sure the dairy population was expanding somewhat in this region during the period covered by these data and an expanding population naturally has a more youthful age distribution than a static one, unless there are some compensating changes in the death rates at various ages. In small part this may explain the relative scarcity of mature and old cows.

SUMMARY

1. The mean age at which 570 registered heifers in Iowa Cow Testing Associations first freshened was found to be 27.1 months but the distribution was decidedly skew with the mode at 24 months. More than half of them freshened before they were 25½ months old. These ages were computed as the last full month attained before the freshening day and therefore are about 15 days less than the actual ages.

2. Some significant differences were found among the different breeds studied. Nearly 73 per cent of the data concern Holsteins.

3. Differences found from herd to herd were large enough to show a variation in management policy in this respect or an effective difference in herd environment.

4. Heifers fed liberally enough to approach advanced registry conditions and heifers fed poorly calved later than those which received about average feeding, but this relation is too slight to be practically important and even its statistical significance is doubtful.

5. The numbers of cows calving at various ages show a distinct cycle, the peaks being twelve months apart and beginning at 24 months. This cyclical distribution should be taken into account in studying the relation of other variables to age at calving.

6. This study is a survey of actual breeding practice among Iowa breeders of purebred dairy cattle. Whether breeding for such early freshening has more desirable than undesirable consequences, everything considered, is something we hope to investigate at an early date.

REFERENCES

- (1) BEARD, LYNN H. Relation of bovine age to season of calving. Unpublished thesis, Iowa State College. 1933.
- (2) ECKLES, C. H. Dairy cattle and milk production. The Macmillan Company, New York, p. 334. 1931.
- (3) FISHER, R. A. Statistical methods for research workers. 4th edition. Oliver and Boyd. Edinburgh. 1932.
- (4) GAINES, W. L., AND SHAW, D. D. Growth and senescence in Red Danish cows as measured by the rate of milk secretion. Jour. Agr. Res. 37: 169-180. 1928.
- (5) HENRY, W. A., AND MORRISON, F. B. Feeds and feeding. 19th edition. The Henry-Morrison Company, Ithaca, N. Y., p. 441. 1928.
- (6) LUSH, JAY L., AND LACY, M. D. The ages of breeding cattle and the possibilities of using proven sires. Iowa Agr. Exp. Sta. Bulletin 290. 1932.
- (7) REED, O. E., FITCH, J. B., AND CAVE, H. W. The relation of feeding and age of calving to the development of dairy heifers. Kansas Agr. Exp. Sta. Bul. 233. 1924.
- (8) SMITH, A. D. B., AND ROBISON, O. J. The average ages of cows and bulls in six breeds of cattle. Jour. Agr. Sci. 21: 136-149. 1931.
- (9) TURNER, C. W. The influence of age at first calving on milk secretion. Missouri Agr. Exp. Sta. Res. Bul. 164. 1932.

THE APPARENT VISCOSITY OF ICE CREAM

I. THE SAGGING BEAM METHOD OF MEASUREMENT.

II. FACTORS TO BE CONTROLLED. III. THE EFFECTS OF MILKFAT, GELATIN AND HOMOGENIZATION TEMPERATURE

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INTRODUCTION

The purpose of this paper is to describe the development and application of the sagging beam method of viscosity measurement to ice cream and to show the effect of certain variables, such as milkfat concentration, gelatin concentration and homogenization temperature upon the viscosity of ice cream.

In ice cream work, particularly from the scientific point of view, there is need for the development of methods for measuring the physical properties of ice cream. Such measurements will serve several purposes. They will define the properties of the finished product and they may be used as a tool to get an insight into the effect of variations of manufacture and the mechanism of their action, as well as furnishing a basis for the general improvement of ice cream. Another point that must not be overlooked is that such measurements may be used to record permanently the properties of certain ice creams, when, with the present day methods of testing by taste, as soon as the control sample is destroyed, direct comparison with that series of tests becomes impossible.

Of the physical properties of ice cream that should be measureable, consistency quite naturally suggests itself, not only because it may be determined, but also because it is undoubtedly one of the properties of ice cream upon which consumer preference depends. Sagging beam experiments in connection with the rheological properties of ice cream have shown that ice cream will flow under comparatively low stresses and that for the purposes of this paper it may be considered as a viscous substance. The following discussion deals, then, with the apparent viscosity of ice cream as measured by the sagging beam method, especial emphasis being placed upon the rôle which milkfat and gelatin play in the physical quality of ice cream as demonstrated by viscosity measurements.

The usual method for measuring the viscosity of a fluid mass is to force it under pressure through an orifice and determine the amount delivered in a given time under certain standard conditions, or to use an instrument

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of the MacMichael type employing a torsion disc. The first method is useless for our purpose since ice cream is a whip and any considerable force placed upon it will materially alter the properties of the mass by breaking up the structure. The second method requires the fluid to flow freely, and hence cannot be used with ice cream. Under these circumstances, the sagging beam method of viscosity measurement suggests itself as being applicable to ice cream.

I. THE SAGGING BEAM METHOD OF MEASUREMENT

The sagging beam method of viscosity measurement was developed by Trouton (1) and was used by him in the determination of the viscosity of pitch and substances of that character. More recently it has been used by Bingham (2) to measure the consistency of cement and of cement-stone-mortar. The method is simple and consists in the determination of the rate of sag of a beam of the substance under investigation when such factors as length and diameter, as well as the weight of the beam are known.

This method, as we have developed it for the measurement of the viscosity of ice cream, is as follows: The ice cream is drawn from the freezer, transferred to the hardening room, and poured into bottomless metal boxes placed upon metal plates. When the beam is to be cut, (usually after the ice cream has hardened one week, as will be shown later) the box of ice cream is torn from the plate and placed upon a wooden block resting upon the bed of a blacksmith's drill press, where the beam is drilled out with a tool of the cork borer type, the cutting edge of which is slightly smaller than the barrel, so that the beam will not bind within the tool. A cylindrical beam $4\frac{3}{4}$ inches long by $\frac{3}{4}$ inch in diameter is obtained in this manner. The beam is then taken to a constant temperature room, held at a temperature of -8° C. and after a period of one hour, in which the beam has reached the temperature of the room, it is placed upon aluminum blocks grooved to receive it and clamped in place by means of small lead weights shaped to fit it. The weight of these clamps actually rests upon the aluminum blocks so that the beam is held in place without crushing. The blocks are a few inches high and rest upon glass plates. The blocks are provided with two grooves upon the upper side and when held in a series of seven, at the proper intervals, by means of a brass rod, provide support for six beams at one time. Plate I shows some beams upon the blocks after sagging about three hours.

After the beam is in place, the distance from the glass plate to the bottom of the center of the beam is measured periodically by means of dividers and this measurement transferred directly to graph paper by pricking the surface, the sag, of course, being plotted against time. The use of a white background is essential while making the measurements with

the dividers. Following this procedure an unalterable record is made as the measurement progresses.

For the purpose of this paper all beams have been measured in duplicate or triplicate and no measurements have been accepted without obtaining satisfactory checks. Each entire experimental series has also been repeated at least once to make sure that the conclusions are valid and that possible errors have not been overlooked.

Trouton (1) has shown that the absolute viscosity of a substance may be calculated from the rate of sag of a beam by the following formula:

$$\eta = \frac{5gmL^3}{1152 RI}$$

where η is the viscosity, g is the gravity constant, m the mass and L the length of the beam between supports. R is the rate of sag of the beam and I the moment of inertia of a cross section of the beam. I is equal to $.049D^4$ where D is the diameter of the beam.

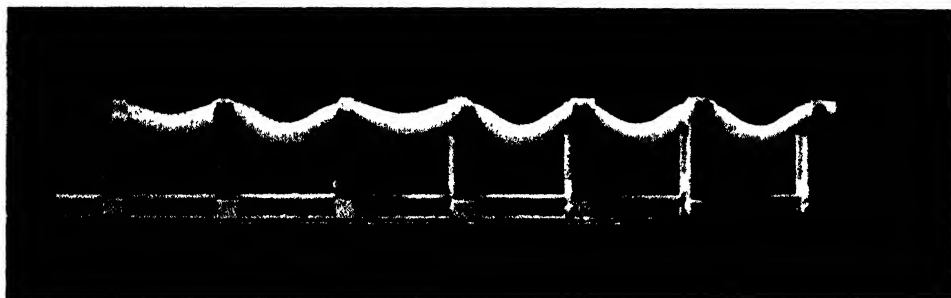


PLATE I

Ice cream beams after the completion of a series of viscosity measurements. Starting at the left, samples 1 and 3 are duplicates and contain no gelatin; samples 2 and 5 are duplicates and contain 1/10% gelatin; samples 4 and 6 are duplicates and contain 3/10% gelatin.

This reduces in the case of our ice cream beams at 100 per cent overrun to

$$\eta = \frac{21000}{R_1} \quad \text{or} \quad \frac{8268}{R_2}$$

where R_1 is the rate of sag in centimeters per second and R_2 the rate of sag in inches per second.

The calculated apparent viscosity, in terms of centipoises, in the case of ice cream is large. A beam of 100 per cent overrun ice cream that would sag at the rate of 2.54 centimeters or an inch in 400 minutes would have an apparent viscosity of 19 billion 800 million (1.98×10^{10}) centipoises.

In our work we have adopted the procedure of comparing the rate of sag of the different specimens of an experimental series directly with the

control. This is desirable because, while absolute duplication of viscosity measurements in specimens made from identical materials is possible, similar ice creams made from day to day vary to some extent in the absolute value of their viscosities. This may be due to the physical variations in the cream and condensed milk used in making the ice cream and is a field for further systematic study.

Types of Sag-Time Curve

When the sag of the ice cream beams is plotted against time, two types of curves may be obtained, as shown in figure 1.

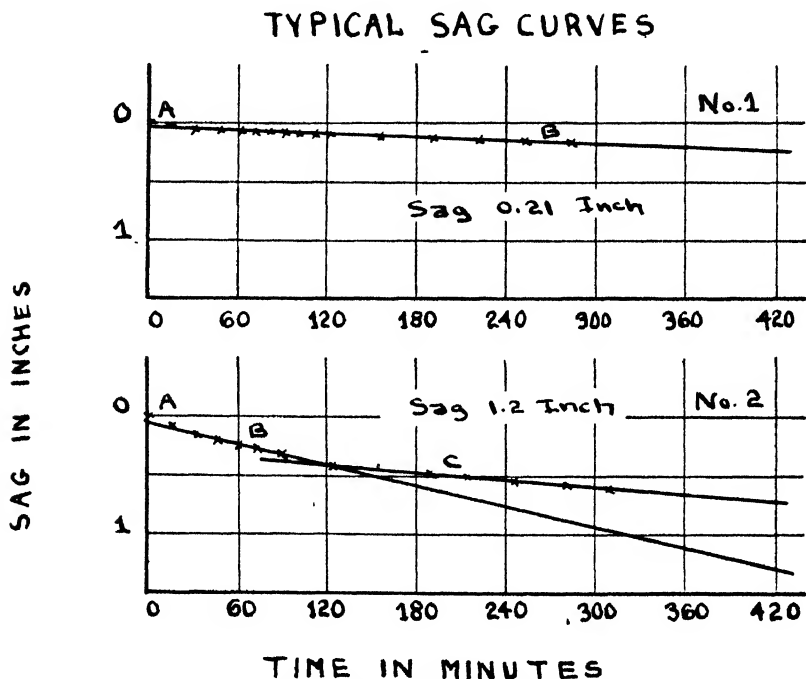


FIG. 1. TYPICAL CURVES OBTAINED BY PLOTTING SAG OF ICE CREAM BEAMS AGAINST TIME

The first curve represents the rate of sag of a rather viscous ice cream in which the sag is small, and, after the beam has adjusted itself to the supports, the sag is proportional to the elapsed time and the points may be joined by a straight line, the slope of which gives information from which viscosity may be calculated. This curve, then, is made up of two parts, the initial sag (A) probably due to the adjustment of any strain imposed upon the beam in placing it upon the supports, and probably also connected with the elastic properties of the beam; and the second part (B) the rate of sag of which, following the precedent of Trouton, we have used to calculate viscosity. This curve has been considered to be the first part

of the curve of the second type as shown in figure 1. This second curve is the type obtained from an ice cream much less viscous than the first, and we see that the rate of sag is slowed appreciably after the beam has sagged, in this case, about half its diameter. It is the second or (B) portion of this curve that gives the viscosity data here, although it seems that both the initial sag (A) and the third part of the curve, the (C) portion, must give some indication of certain properties of ice cream as yet undetermined. A field for further experimentation has here been opened up.

In the work reported here the determination of the slope of the line representing the rate of sag is facilitated by the extrapolation of the line across the entire width of the graph paper, which usually means the determination of sag which would have occurred in 400 minutes had the sag remained constant in rate throughout that interval.

In some sag curves the three portions of the curve are not so apparent as those illustrated in the second curve of figure 1. In such curves the points of the third portion are easily recognized and a line drawn through them indicates the point which is to be considered as the last point of the second portion of the curve. From this point, working backwards, a straight line is drawn through all the points that will fall on the line. The slope of this line is used to determine viscosity.

II. FACTORS TO BE CONTROLLED

The standardization of the sagging beam method for the measurement of the viscosity of ice cream for such purposes as, for instance, the determination of the effects of variations in composition, necessitates the investigation of the influence of certain variations in procedure.

The viscosity of ice cream is affected quite markedly by temperature. A lowering of the temperature of an ice cream sample by a few degrees may double or treble the viscosity of that sample. For comparative studies, however, we have worked at only the one temperature, $-8^{\circ}\text{C}.$, since a brief series of tests showed the apparent relative viscosities of different ice cream samples in a series to be approximately the same at all temperatures at which the sagging beam measurements were possible.

The temperature of $-8^{\circ}\text{C}.$ was used because it approximated the temperature at which ice cream is usually served and also because it proved a convenient temperature to work at.

It was found that ice cream samples placed in the hardening room required from two to four days to reach equilibrium. Therefore the procedure was adopted of making measurements at a period of one week from the date of freezing. Difficulties due to the presence of air holes in the ice creams were encountered at first, but this trouble was overcome by reducing the speed of the freezer as the sample portions were drawn.

Two important factors to question were the effect upon viscosity of overrun and temperature of drawing. A considerable number of experiments show that, for a normal fat mix and one low in fat, overruns between the limits of 50 and 100 per cent do not affect the rate of sag. This is also true of overruns between 60 and 100 per cent in high fat mixes. This does not mean that viscosity is independent of overrun, but rather that it is inversely proportional to overrun. This is explained by the fact that viscosity is proportional to the mass divided by the rate of sag. If the mass of one specimen is greater than that of a second specimen, and the rate of sag of both specimens is the same, then the first must have the greater viscosity.

The effect of temperature of drawing is shown by the curves of figure 2.

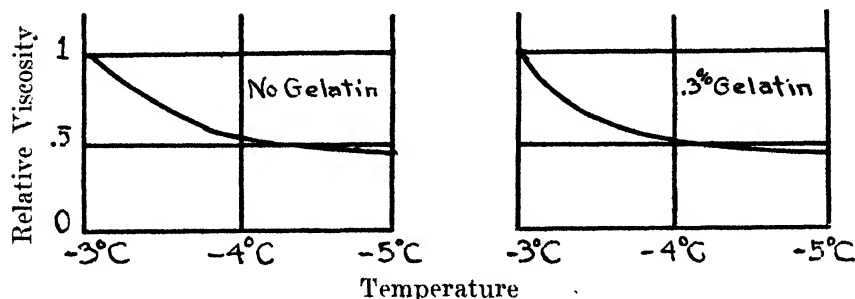


FIG. 2. VARIATION OF ICE CREAM VISCOSITY WITH TEMPERATURE OF DRAWING.

The viscosity of ice cream as measured by the sagging beam is less with lower temperatures of drawing, probably because the ice crystals are smaller with lower drawing temperatures. For work on crystal size see Cole (3), Turnbow (4) and Dahlberg (5). It is interesting that the two curves, the one for an ice cream made without gelatin and the other for an ice cream made with gelatin, are practically identical. Although the absolute viscosities of the samples containing gelatin are lower.

III. THE EFFECTS OF MILKFAT, GELATIN AND HOMOGENIZATION TEMPERATURE

The effect of milkfat upon the viscosity of ice cream is marked as shown in figure 3.

In order that milkfat concentration should be the only variable in the ice cream made from these mixes, the milk-solids-not-fat, water and sugar content was maintained in the same proportion as in a mix containing 64 parts water, 10 parts milk-solids-not-fat and 14 parts sugar. The milkfat was varied through the range of 3, 6, 9, 12, 15, 18 and 21 parts as compared with 88 parts of the other ingredients.

At the time of drawing figure 3 it seemed desirable to use the viscosity of the 3-parts-fat sample as the control due to the fact that under ordinary

conditions of freezing it was found impossible to keep the overrun of samples made up without milkfat within any reasonable bounds. However, a later experiment, wherein the overrun of a mix made without milkfat was controlled by the expedient of overloading the freezer, shows that the figures given above are valid.

It may be seen from the plot that increase in fat content first increases the viscosity of ice cream, then decreases it markedly to a minimum between the ratios of 12, 15 and 18 parts fat and then increases the viscosity at 21 parts. Of these ice creams the 18 parts fat ice cream had the best body and texture as determined by taste. It is evident that with increasing fat content the viscosity of ice cream is first increased through a binding or mass

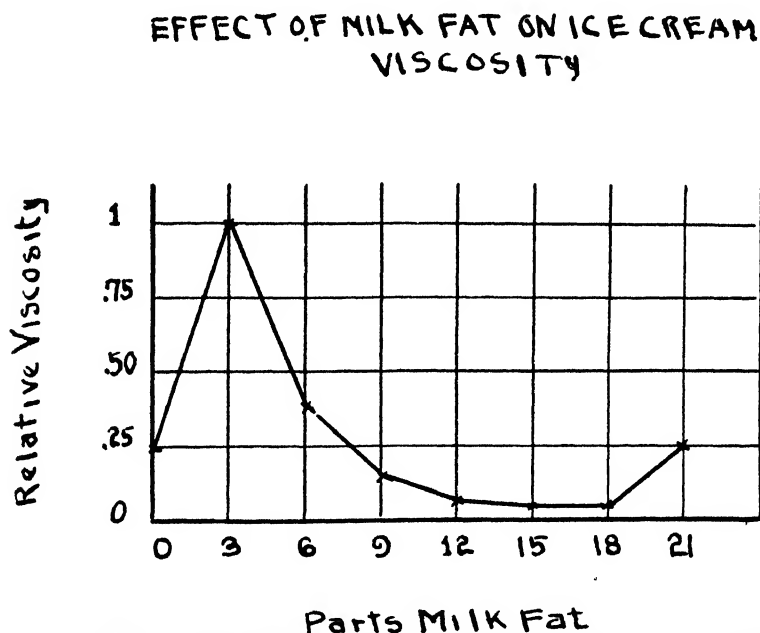


FIG. 3. EFFECT OF MILKFAT CONTENT UPON THE VISCOSITY OF ICE CREAM

effect of the milkfat. Then with increasing quantity of fat the protective effect on the ice crystals or a lubricating action results in lower viscosity simultaneously with better texture. Finally the mass effect of the large amount of fat becomes evident and the viscosity increases. A further analysis of the curve shows that in this curve, and it is also true of some that follow, there are possible three different ice creams of the same viscosity but widely varying quality. This means that a simple determination of the viscosity of any ice cream can not serve to indicate the quality of that ice cream. It is equally true, however, that when any factor such as fat content or gelatin content is varied in a series of ice creams, the viscosity curve indicates the changes in quality of the ice creams, and it has been found that

the ice cream of the most desirable quality was always either one at the lowest possible viscosity or one a short way along the viscosity up-curve where a little more body was to be had than in the ice cream possessing lowest possible viscosity. Viscosity then becomes an instrument for recording in absolute terms changes in the comparative qualities of ice creams that have heretofore been unmeasurable and incapable of adequate description. It is a tool for the research worker rather than the manufacturer unless it so happens the latter should find the measurement of value in checking the standard of the product from time to time.

Before considering the effect of gelatin upon the viscosity of ice cream let us study this curve (Fig. 3) more closely. The points to be noted are that the addition of fat, in not too great an amount, would decrease the viscosity of an 8 parts fat mix and also that in the usual range of fat variation found in commercial ice cream viscosity changes are not so great as in the lower fat ranges. The absolute viscosities are also the lowest possible.

Effect of Gelatin Upon Ice Cream Viscosity

In figure 4 the curve shows the effect of variations in the amount of

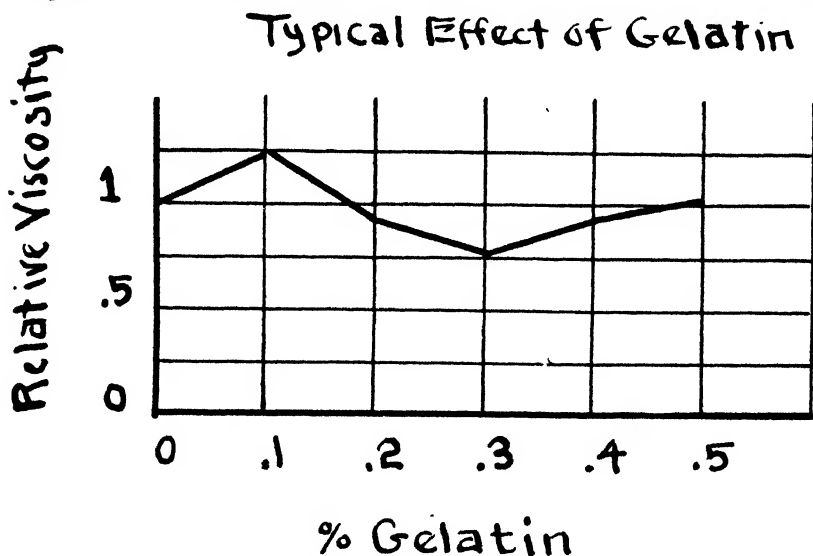


FIG. 4. TYPICAL EFFECT OF VARIATIONS IN THE AMOUNT OF GELATIN UPON THE VISCOSITY OF ICE CREAM

gelatin upon the viscosity of ice cream. This is a typical curve, characterized by a minimum, for mixes other than those of high fat content or those homogenized at temperatures higher than usual. The position of the minimum varies with the fat content and homogenization temperature and the minimum vanishes when the fat content is great and the homogenization temperature is sufficiently high.

Here, as in the experiments in which milk fat was varied there is first found a slight increase in viscosity with increased gelatin, then a decrease to a minimum and then a rise. The ice cream having the most desirable body and texture is one on the rising curve, just after the minimum is reached. Here, as in the case of the experiments with milk fat variation, viscosity decreases with improvement in texture. These results are entirely in accord with Dahlberg's observation that gelatin owes its chief value in ice cream to its ability to form a gel. Dahlberg (5) shows that 0.7 per cent gelatin (presumably of good grade) will gel in solution at 0° C. On this assumption, when one considers the concentrating effect of ice separation in ice cream, it seems logical that in the hardening room an initial gelatin concentration of 0.1 per cent will gel at 0° F. The tentative theory of the above curve, then, is that the gelation of 0.1 per cent gelatin is too weak to interfere with the growth of ice crystals, but that at slightly higher concentrations interference results in lower viscosity in spite of gelation until concentrations of gelatin become such that gelatin actually overcomes the first effect and a higher viscosity product results. It is of course apparent from data recorded elsewhere in this paper that factors other than simple concentration of ingredients enter in, such as homogenization temperature, etc., so the complete theory of ice cream viscosity is not so simple as indicated above.

Effect of Gelatin and Homogenization Temperature Upon Ice Cream Viscosity

In considering the effect of gelatin upon the viscosity of ice cream, the question naturally arises as to what is the effect of homogenization temperature and pressure. The next three figures show the effect of variation of gelatin content simultaneously with homogenization temperatures of 53°, 63°, 73°, and 83° C. upon the viscosity of ice creams containing 8, 12, and 16 per cent fat. The effects of homogenization pressure will not be reported upon in this paper.

The effects of gelatin concentration and homogenization temperature variation upon the viscosity of mixes of low milkfat content, (8% butterfat, 12% milk-solids-not-fat and 14% sugar) are shown in figure 5.

It will be noted that in ice cream made from mixes homogenized at 53° C., the viscosity decreases through 0.5 per cent gelatin. Actually later experiments showed it to decrease through 0.75 per cent gelatin with a slight viscosity increase at 1.0 per cent gelatin. The mix homogenized at 63° C. shows a decrease in viscosity through 0.4 per cent gelatin, then a rise. Here the 0.5 per cent gelatin content gave the best ice cream when considering body and texture. At 73° C. homogenization temperature the minimum is reached at 0.3 per cent gelatin and at 83° C. the minimum is either very slight or missing entirely. We see then that with higher homogenization

temperatures there is apparently less gelatin required before gelation overcomes the protective effect. In this sense higher homogenization temperatures replace gelatin. Referring back to figure 3 it will be seen that the addition of milkfat to an 8 per cent fat mix should, in moderate quality, reduce its viscosity. In the mixes here we find gelatin, under normal conditions, doing the same thing. Gelatin, then, in a way appears to act in ice cream as does fat in its effect upon viscosity.

8% FAT ICE CREAMS

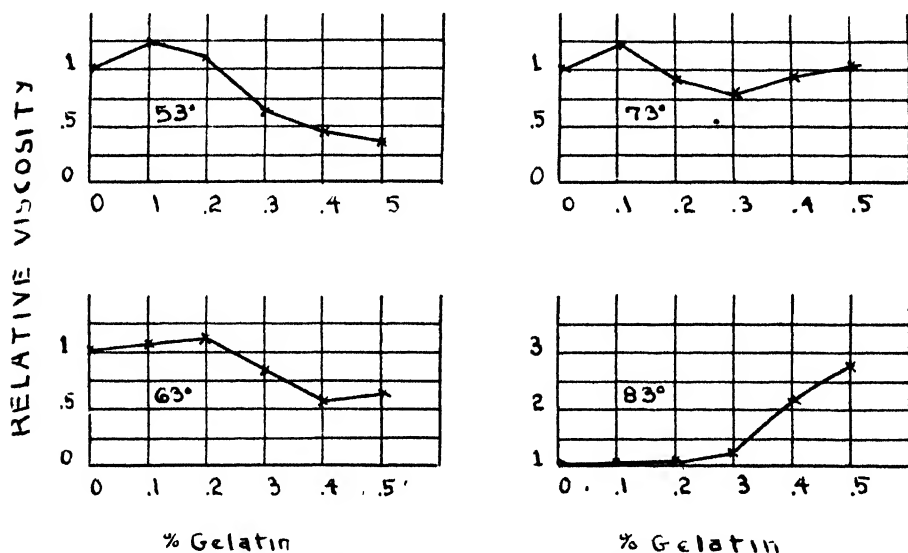


FIG. 5. CURVES SHOWING THE EFFECT OF VARIATIONS IN THE AMOUNT OF GELATIN UPON THE VISCOSITY OF ICE CREAM MIXES OF LOW FAT CONTENT

HOMOGENIZED AT DIFFERENT TEMPERATURES

53° C. homogenization.

73° C. homogenization.

63° C. homogenization.

83° C. homogenization.

(Note change of coordinates)

In similar data for mixes containing 12 per cent fat it is apparent, as might be expected, that the tendency for gelatin to decrease viscosity is not as marked as before. The tendency to gelation is greater, however, and, to get a given body, less gelatin is required in this series of mixes. There is, as before, the tendency for higher temperatures of homogenization to reduce the quantity of gelatin that must be present to increase the viscosity of ice cream.

Another similar set of curves of the viscosity of ice creams of 16 per cent milkfat content is shown in figure 7. Here, as indicated in figure 3, there is no tendency for the gelatin to reduce viscosity, but there is, particularly at the higher temperatures, a marked gelation effect. As in the

12% FAT ICE CREAMS

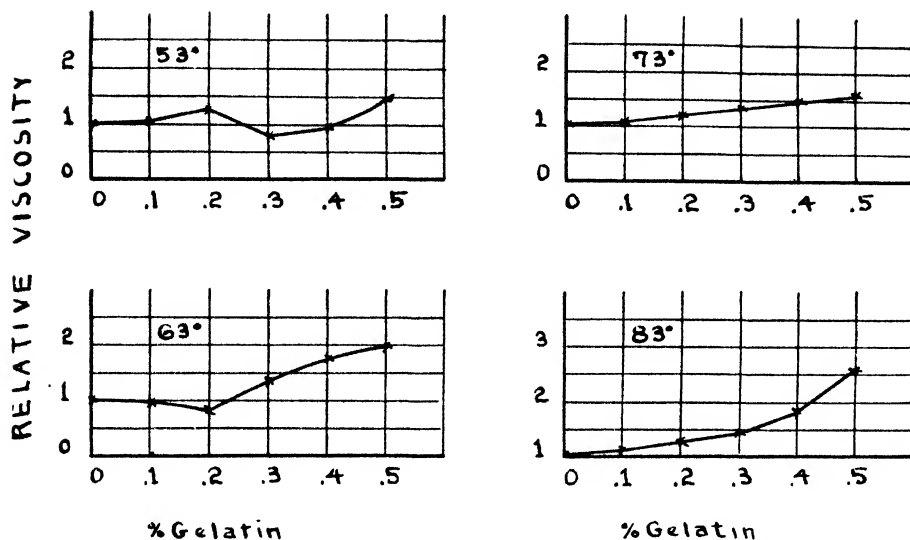


FIG. 6. CURVES SHOWING THE EFFECT OF VARIATIONS IN THE AMOUNT OF GELATIN UPON THE VISCOSITY OF ICE CREAM MIXES OF MEDIUM FAT CONTENT

HOMOGENIZED AT DIFFERENT TEMPERATURES

53° C. homogenization.

73° C. homogenization.

63° C. homogenization.

83° C. homogenization.

16% FAT ICE CREAMS

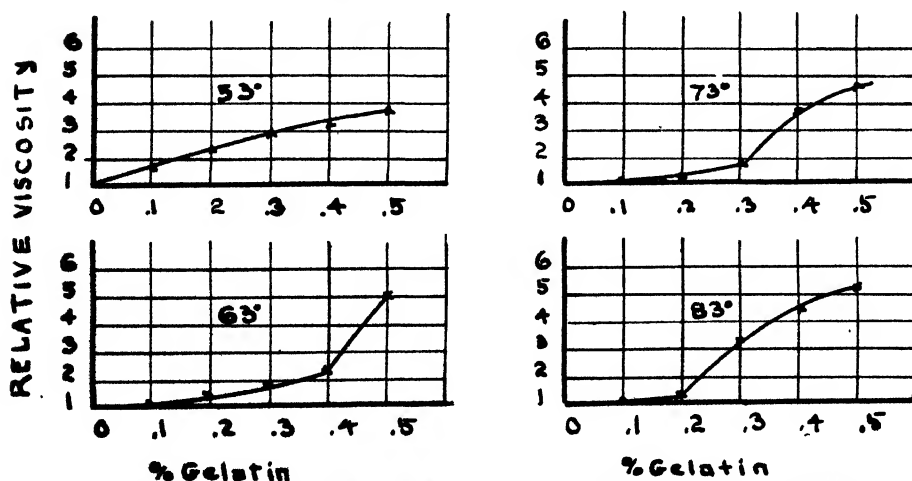


FIG. 7. CURVES SHOWING THE EFFECT OF VARIATIONS IN THE AMOUNT OF GELATIN UPON THE VISCOSITY OF ICE CREAM MIXES OF HIGH FAT CONTENT

HOMOGENIZED AT DIFFERENT TEMPERATURES

53° C. homogenization.

73° C. homogenization.

63° C. homogenization.

83° C. homogenization.

other curves, higher temperatures seem to lower the amount of gelatin necessary to cause an up-turn in the viscosity curve.

We can conclude from the data given above that milkfat and gelatin seem to be working both independently and together in some way to produce the same general effects upon the viscosity of ice cream and that high homogenization temperature lowers the amount of either material necessary to produce the same effect.

SUMMARY

It has been shown that the sagging beam method of viscosity measurement can be used to determine the rheological properties of ice cream, and that ice cream may for all practical purposes be considered a viscous material.

A detailed study has been made of those factors of normal manufacture, such as overrun, temperature of drawing, etc., which would affect the viscosity of ice cream, so that a standard procedure could be developed for determining ice cream viscosity.

The effect of milkfat concentration and gelatin concentration upon the viscosity and quality of ice cream has been studied. It is shown that they in some way act to produce the same general effect upon the viscosity of ice cream. Increasing temperature of homogenization tends to reduce the quantity of either material necessary to produce the same general effect.

While it is evident that the absolute viscosity value of an ice cream is not a direct measure of quality, it is shown that, in a series of ice creams in which one factor is varied, viscosity is an indication of changes in quality and of the physical action of that factor in ice cream.

ACKNOWLEDGMENTS

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REFERENCES

- (1) TROUTON, F. T. On the coefficient of viscous traction and its relation to that of viscosity. *Proc. Royal Soc. London A* 77: 426. 1906.
- (2) BINGHAM, E. C. The rheological properties of cement and cement-stone-mortar. *Physics* 4: 88. 1933.
- (3) COLE, W. C. Studies in freezing ice cream. *Ice Cream Field* 16 (5): 15. 1930.
- (4) TURNBOW, G. D. Science shows industry new leads in the processing of ice cream. *Ice Cream Trade Jour.* 24 (8): 50. 1928.
- (5) DAHLBERG, A. C. The texture of ice cream. *Tech. Bulletin No. 111, Geneva, N. Y., Exp. Sta.* 1925.

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THE COLOR IMPARTED TO COFFEE BY CREAM TREATED IN VARIOUS WAYS

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While making a comparison of bottled cream distributed by several competing dairies it was observed that equal volumes of the creams did not always impart to coffee a constant color. This observation, together with reports from consumers that some creams "went further" in coffee than others, led to an investigation of the factors concerned with the color of coffee and cream mixtures.

There are many points which could be included in such an investigation such as the type of coffee used, the manner in which the coffee is prepared and creamed, the influence of temperature of the coffee, a study of the actual shades of brown produced in the coffee-cream mixtures under various conditions, and the influence of the cream itself. However, it is the purpose of this paper to discuss only those factors over which the dairy plant has control, *i.e.*, the cream itself, how its coloring power varies with composition, and the manufacturing processes to which it may be subjected in a bottling plant.

APPARATUS AND METHOD USED TO MEASURE COLORING POWER OF CREAM

The average coffee drinker who flavors and colors coffee usually adds sufficient volume of milk, cream, evaporated milk, or other dairy products to impart a rich golden yellow-brown color to the coffee. To imitate as closely as possible the procedure followed by the average person in creaming coffee, a method of testing cream was developed which determined the volume of any given cream required to impart a constant yellow-brown color to a given volume of coffee of standard strength and temperature.

Preparation of standard coffee solution—One coffee solution was used in all of this work. This was prepared by swirling for 5 minutes in 5 gallons of water at 200° F. a cheese-cloth bag containing 2.2 pounds of medium ground coffee. After the coffee solution had been filtered through a milk filter it was transferred to quart and pint milk bottles. These were filled about two-thirds full and covered with heavy tin foil. The bottles

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of coffee were then sterilized in an autoclave under 15 pounds of steam pressure for 25 minutes, cooled and stored at room temperature until required.

Color standard—Webb and Holm (1) found the Munsell system of measuring color to be satisfactory in their studies on the color of evaporated milk. By means of rapidly revolving discs of colored paper of selected colors, any given shade of color can be produced. Use was made of this apparatus and three colored discs, yellow, red, and black, in establishing a constant golden yellow-brown standard with which the coffee-cream mixtures could be compared. The shade of color selected was that produced when 1 part of 20 per cent cream is added to 10 parts of average coffee.

How the test was made: A series of large test-tubes holding 100 cc. of the standard coffee solution was prepared. These were heated as required to a temperature of 180–200° F. in a boiling water bath. The coffee was then poured into a small white cup containing 10 cc. of cream at 40–45° F. The coffee-cream mixture was placed beside the revolving color standard and the color compared with that of the standard through an eye-piece assembly which had been removed from a Bausch and Lomb colorimeter of the Duboseq type.

A constant light source was maintained by means of a blue “daylight” frosted lamp installed in such a way that the same amount of light fell at about a 45 degree angle on the surface of both the revolving color standard and the coffee-cream mixture. All other sources of light were carefully eliminated.

The coffee-cream mixture was stirred very gently every few seconds to insure uniformity of color, while a comparison of the colors was being made. Readings were always made in less than 30 seconds to avoid variations due to a decrease in temperature.

If it was found that the color standard was darker than the coffee-cream mixture another test was made using 9.5 cc. of cream. If the color standard was found to be of a lighter shade than the coffee-cream mixture then 10.5 cc. of cream was used in the second test. These tests were repeated using more or less cream as indicated until the color standard was matched.

The number of cc. of cream required to produce the standard color in 100 cc. of coffee, when measured by the method described above, was taken as the coloring power of the cream.

VARIATIONS IN COMMERCIAL PRODUCTS

In order to demonstrate that creams offered to the retail trade vary considerably in their power to impart a given color to coffee, samples of “coffee” cream and “whipping” cream, bottled by thirteen competing

dairies in Baltimore, were purchased retail on the same day. These were examined for acidity, percentage of fat and solids-not-fat, and the amount of cream required to color 100 cc. of coffee. The data are summarized in table 1.

TABLE 1

Variations in cream purchased on the same day from stores and wagons of thirteen dairies competing in the Baltimore area

DAIRY	PURCHASED AS "COFFEE CREAM"				PURCHASED AS "WHIPPING CREAM"			
	Acidity	Fat	Solids-not-fat	Amt. of cream to color 100 cc. coffee	Acidity	Fat	Solids-not-fat	Amt. of cream to color 100 cc. coffee
	%	%	%	cc.	%	%	%	cc.
A	0.16	19.5	8.65	10.4	0.25	24.5	9.20	v. sl. curdled
B	0.11	26.5	6.83	8.9	0.11	36.0	5.56	7.1
C	0.14	20.0	8.12	9.6	0.15	30.0	7.71	8.1
D	0.10	23.0	6.46	9.8	0.09	30.0	9.16	7.9
E	0.10	24.0	6.49	9.7	0.10	32.0		8.1
F	0.11	25.5	7.73	8.9	0.10	29.5	5.39	8.3
G	0.08	21.0	7.69	10.3	0.12	31.5	6.08	8.1
H	0.11	22.0	7.49	10.0	0.10	36.5	4.58	7.4
I	0.11	33.5	6.12	7.7	0.10	33.0	6.67	7.6
J	0.18	20.0	10.41	9.5	0.11	28.5	5.85	8.5
K	0.12	17.5	7.21	10.6	0.10	37.5	5.28	7.1
L	0.24	19.5	13.70	v. sl. curdled	0.15	33.5	8.23	7.1
M	0.16	23.0	7.09	9.7	0.15	34.5	5.70	7.3

It will be seen from the information in table 1 that vast differences exist between products offered to the public by the dairies of Baltimore although bottled and sold under one of two types of cream. Variations of this magnitude would easily be noted by the average coffee drinker in a restaurant where a measured amount of cream is served with coffee and by the more observant consumer who adds cream to coffee from a small pitcher.

Dairies who do not practice close standardization may distribute cream which varies considerably from day to day. This is seen from the data presented in table 2. Dairy C could be depended upon to supply a uniform product, while Dairy B, probably through lack of standardization, was not selling a constant product.

PLANT PRACTICES AND THEIR INFLUENCE ON THE COLOR IMPARTED TO COFFEE

The fat content of the cream. The data presented in table 1 indicate that the fat content of the cream is a factor controlling the color of coffee and cream mixtures. In order to show how the fat content affects the color a series of creams containing various percentages of fat was prepared by standardizing raw 40 per cent cream with skim milk. These creams were

TABLE 2

Daily variations in "coffee cream" from two competing dairies

DATE 1934 MAY	AMOUNT CREAM REQUIRED TO COLOR 100 CC. COFFEE	
	Dairy B	Dairy C
	cc.	cc.
17	9.0	9.7
18	9.0	9.7
19	8.5	9.6
21	9.2	9.6
22	8.9	9.6
25		9.7
26	9.1	9.6
27	9.1	9.7
29	8.6	9.7

pasteurized, cooled and aged one day at 40° F. before testing. The data procured are summarized in table 3.

The milk-solids-not-fat content of creams prepared in this manner varies inversely with the fat content and since it also appears from table 1 that the milk-solids-not-fat has an influence on the color of cream and coffee, another series of creams was prepared as above but with a constant milk-solids-not-fat content. This was accomplished by adding skim condensed milk to the heavier creams. The constant amount of milk-solids-not-fat selected was 7.0 per cent, which is close to the average quantity found in 20 per cent cream. Table 3 also contains the data obtained with this series of cream.

A study of table 3 shows that the color-producing power of cream varies inversely with the fat content of the cream. The coffee drinker therefore requires less of a rich cream than of a thin or low-fat cream.

The milk-solids-not-fat content of the cream. It appears from tables 1 and 3 that the milk-solids-not-fat content of the cream has an influence on

TABLE 3

The influence of fat content of cream on its ability to color coffee

CONTENT CREAM	AMT. CREAM TO COLOR 100 CC. COFFEE	
	Normal solids-not-fat in cream	Constant solids-not-fat in cream (7.0%)
%	cc.	cc.
15	11.1
20	10.0	10.0
25	9.0	9.0
30	8.1	8.0
35	7.0	6.8
40	5.9	5.7

the color imparted to coffee by cream. This inference is substantiated by the data presented in table 4, which gives the observations made on a series

TABLE 4
The influence of milk-solids-not-fat content on ability of 20 per cent cream to color coffee

ADDITIONAL MILK-SOLIDS-NOT-FAT IN CREAM	AMT. CREAM TO COLOR 100 CC. COFFEE	
	Solids from skimmilk powder	Solids from skim condensed milk
%	cc.	cc.
0.0	10.0	10.0
0.5	9.5	9.6
1.0	9.0	9.1
1.5	8.8	8.8
2.0	8.5	8.6
2.5	8.3	8.3
3.0	8.0	8.1

of 20 per cent creams with increasing milk-solids-not-fat contents. The milk-solids-not-fat content was increased in one case with dry skimmilk and in the other with skim condensed milk. These products were added prior to pasteurization.

Table 4 shows that as the solids-not-fat content of cream is increased its power to color coffee is also increased, so that when cream is fortified with milk-solids-not-fat less cream is required in coffee.

The influence of homogenization. Cream is sometimes homogenized at low pressures to improve the body. That this practice has an influence on the ability of cream to color coffee is shown by the data presented in table 5. This observation confirms that of Mohr and Barfuss-Knochendöppel

TABLE 5
The influence of homogenization on the ability of 20 per cent cream to color coffee

TREATMENT GIVEN CREAM	AMT. CREAM REQUIRED TO COLOR 100 CC. COFFEE
	cc.
Unhomogenized	10.0
Homogenized at 120° F.—	
at 400 lbs. pressure	9.4
at 600 lbs. pressure	9.2
at 800 lbs. pressure	9.1
	(sl. curdled)

(2) who showed that homogenization of 10 per cent and 15 per cent cream increased the "Weiskraft."

When cream is homogenized it appears whiter in color than the same cream unhomogenized (3). The greater the homogenizing pressure the

greater the whitening power of the cream (2). This indicates that the fat globule size is responsible for the difference noted between homogenized and unhomogenized cream.

The influence of pasteurization. A goodly portion of the cream sold in the United States is pasteurized. From table 6 it will be seen that pas-

TABLE 6
The influence of pasteurization on the ability of cream to color coffee

FAT CONTENT OF CREAM	TREATMENT	AMOUNT CREAM REQUIRED TO COLOR 100 CC. COFFEE
%		cc.
20	Raw	10.0
	Pasteurized at	
	150° F. for 30 min.	10.0
	185° F. for 15 sec.	9.8
30	Raw	8.2
	Pasteurized at	
	150° F. for 30 min.	8.2
	185° F. for 15 sec.	8.0
40	Raw	6.1
	Pasteurized at 145° F. for 30 min.	6.1

teurization at 150° for 30 minutes has no effect on the coloring power of the cream. Flashing cream to 185° F. improved slightly the ability of the cream to color coffee. Mohr and Barfuss-Knochendöppel (2), in addition to making a study of the influence of pasteurization, showed that boiling the cream further increased the whitening power of cream.

By far most of the pasteurized cream sold for table use is treated by the holder method, hence it may be said that pasteurization has no influence on the ability of cream to color coffee.

The influence of aging. Many dairies age cream one or more days after pasteurizing to improve the body of the cream. In order to study the effect of this practice on the coloring power, samples of pasteurized 20 per cent cream were aged for several days at 40° F.

It was found that aging of the cream had no effect on the color imparted to coffee by the cream.

The influence of heat-treating cream. Hening and Dahlberg (4) have shown that when chilled cream is carefully heated to a temperature between 80° and 90° F. and then slowly cooled its viscosity is increased. This may be done either in a vat by the batch method or continuously in tubu-

lar equipment. Table 7 gives the data on two typical creams treated by the batch method.

TABLE 7
The influence of giving cream a temperature treatment to increase its viscosity on ability of cream to color coffee

FAT CONTENT	TREATMENT GIVEN CREAM	AMOUNT CREAM REQUIRED TO COLOR 100 CC. COFFEE
%		cc.
20	Control—normal cream	10.0
20	Heated slowly to 86° F., cooled slowly	10.3
30	Control—normal cream	8.2
30	Heated slowly to 85° F., cooled slowly	8.6

It will be seen from table 7 that when cream is given a temperature treatment to increase its viscosity more cream is required to produce a given color in coffee.

The influence of acidity. Observations on several series of creams of different acidities showed that the acidity of 20 per cent cream is not a factor in determining the quantity of cream required to impart a given color to coffee.

SUMMARY

A test was devised for evaluating creams in regard to the color imparted to coffee.

Bottled creams produced by competing dairies in one city were found to vary considerably in their ability to color coffee.

The amount of cream required to impart to coffee a given color decreased when the fat content increased, when the milk-solids-not-fat content increased, and when the cream was homogenized.

Pasteurization of cream by the usual holding method has no influence on the color of coffee-cream mixtures. Flash pasteurizing seems to have a slight influence.

When cream is given a temperature treatment to increase its viscosity it does not color coffee quite as well as the same cream untreated.

Aging of the cream and the development of slight acidity have no influence on the quantity of cream required to color coffee.

REFERENCES

- (1) WEBB, B. H., AND HOLM, G. E. Color of Evaporated Milks. JOURNAL OF DAIRY SCIENCE 13: 25 (1930).

- (2) MOHR AND BARFUSS—KNOCHENDÖPPEL. On the Whiteness of Milk and Milk Products in Coffee. *Molkerei-Zeitung* 75: 1709 (1933).
- (3) DOAN, F. J. The Color of Cows' Milk and Its Value. *JOURNAL OF DAIRY SCIENCE* 7: 147 (1924).
- (4) HENING, J. C., AND DAHLBERG, A. C. A Temperature Treatment for Increasing the Viscosity of Sweet Cream. N. Y. State Agr. Expt. Sta. Tech. Bul. No. 197 (1932).

SOME PHYSICO-CHEMICAL PROPERTIES OF LACTOSE

IV. THE INFLUENCE OF SALTS AND ACIDS UPON THE MUTAROTATION VELOCITY OF LACTOSE

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Lactose contains a free aldehyde group, and therefore it is capable of existing in two different isomeric modifications. When either of these is dissolved in water, it is partially transformed into the other until an equilibrium is reached. The transformation is slow at low temperatures. This fact is of practical importance because the rate of mutarotation sets an upper limit to the rate of solution, or of crystallization of lactose. The fact that the two forms have quite different solubilities is responsible for the recent production of beta lactose on a commercial scale.

Erdmann (11) was the first to observe that the optical rotation of ordinary lactose solutions decreased on standing. He also prepared a new modification of lactose whose optical rotation increased with time. Dubrunfaut (10) also observed the fall in rotation of freshly prepared solutions, and reported that the fall was accelerated by heat. Later, Schmoeger (42) rediscovered what is now known as beta lactose. Although Erdmann's results had been published much earlier, they apparently had not received much recognition (12, 43).

During the latter part of the nineteenth century, a number of papers were published dealing with the mutarotation of sugars. Tanret (46) described three forms of lactose which he called alpha, beta, and gamma lactose. His gamma sugar is now known as beta lactose. The form which he named beta lactose was a mixture of the other two sugars. For a time the equilibrium mixture of mutarotating sugars was quite generally accepted as a distinct modification. Even as late as 1903, we find Roux (41) asserting that the aldoses can exist in three forms.

At the beginning of the twentieth century, several papers appeared which revolutionized ideas regarding mutarotation. Previous to that time, Fischer (13) had suggested that the mutarotation was due to a hydration reaction, and that constant rotation was established only when the sugar was completely hydrated. Trey (47) attributed the mutarotation to some unknown change in configuration. Tanret apparently considered his beta lactose (equilibrium mixture) to be the aldehyde form. Then in 1899, Lowry (25) suggested that the mutarotation was due to the establishment of a dynamic equilibrium between structural isomers, and that the beta

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form of sugars was not a separate individual but was a mixture of the alpha and gamma forms. Later, after the appearance of a paper by Armstrong (2) showing the relation of the alpha and gamma sugars to the alpha and beta glucosides, Lowry (26) published his theory of dynamic isomerism in more detail. He believed that the change from the alpha to the beta form must be a two stage reaction, and that either the aldehyde form, or its hydrate, must be formed as an intermediate product. He also believed that "... apart from its ionizing properties, water is probably directly concerned in the isomeric change of glucose, since this change appears to depend upon a process of reversible hydrolysis." (26, page 1320).

Hudson (16), independently, suggested that the mutarotation of sugars was due to a reversible equilibrium. He published a number of papers dealing with the mutarotation of sugars, particularly of lactose, and discussing the effect of the reversible equilibrium upon the physical behavior of the sugars. (17, 18, 19, 20, 21, 22). Hudson's views differed in one respect from those of Lowry. Lowry postulated an equilibrium between two lactoses with the aldehyde, or its hydrate, as an intermediate, but transitory, form. However, Hudson believed that the mutarotation was due to the reversible reaction,



Hudson believed that hydrated lactose was neither alpha nor beta, but was related equally to each. Lowry (27), however, maintained that the hydrate of glucose was a true hydrate of alpha glucose. More recently, Gillis (14) has claimed also that hydrated lactose is a hydrate of the alpha modification.

In recent years, there has been considerable speculation regarding the actual mechanics of the mutarotation reaction. There is no question about the nature of the end products. The lactone formula is generally accepted, for the alpha and beta sugars, but there is some argument about the nature of the intermediate compounds if such exist. The evidence is largely indirect since it is based chiefly upon a study of the factors which influence the velocity of mutarotation.

Many papers have appeared showing that the mutarotation of lactose, or of sugars in general, is influenced by many factors (51, 52, 44, 45, 47, 48, 38, 49, 26, 27, 19, 37, 1, 33, 50, 39). In general, the mutarotation of sugars is accelerated by an increase in temperature. It is also accelerated by the action of alkalis and acids. Alkalis are more effective than acids, consequently the minimum velocity of mutarotation is not at true neutrality but at pHs below seven. Mutarotation is retarded by alcohols, and by acetone. The action of salts is variable. Some salts have been reported as influencing the mutarotation velocities, but this is commonly attributed to hydrolysis, with a consequent change in pH.

Regarding the mechanics of mutarotation, there are two principal theories. In order to convert alpha lactose to beta lactose, it is necessary only that there be a migration of one proton. Baker believed that this migration might take place directly, without the intervention of any other substance. He has published several papers in support of this theory. (3, 4, 5, 6). Opposed to this view, we have the statement of Lowry, "Catalysis by acids and bases of prototropic changes and of hydraulysis, is interpreted in terms of electrolytic theory according to which a flow of valency electrons through the molecule is produced by bringing a proton donator, and a proton acceptor or a hydroxyl donator, into contact with the two terminals of the labile complex." (30, page 2565). In an earlier paper, he had asserted that, "There is no valid evidence in the literature to prove that mutarotation can take place except by the intervention of water." (28, page 1375). In subsequent papers, this idea was extended as is indicated by the first quotation. Briefly, it is believed that the transfer of a proton is brought about in two or more stages. One proton is withdrawn from one part of the molecule, while another proton is supplied to some other part, thereby keeping the molecule electrically neutral. In a later stage, this process is reversed, but the new proton does not necessarily enter into the same position which was originally occupied by the proton which was withdrawn. In this way, the transformation from one isomer to the other is accomplished. According to this theory, the mutarotation of sugars can occur only in the presence of a substance capable of supplying protons to the molecule and a substance capable of accepting a proton from it. Both a donator and an acceptor must be present. In recent years, largely as the result of studies of systems in non-aqueous solvents, many substances have been found to serve as donators and acceptors of protons. (9, 8, 24, 15). Accordingly, many substances might influence the mutarotation of sugars. This theory that mutarotation can occur only in the presence of a donator and an acceptor of protons has been developed by Lowry (28, 23, 29, 30, 31, 32, 34, 40, 35).

The possibility that a variety of substances might accelerate the mutarotation of lactose seemed worthy of study because the mutarotation velocity of lactose is a factor governing the maximum velocity of crystallization, and of solution. If mutarotation is accelerated greatly by substances normally present in dairy products, then these calculations are not valid. For this reason, an attempt was made to secure data which would indicate the magnitude of the errors which might be introduced by the action of the various milk constituents. At the same time, it was resolved to review, critically, the published data regarding the variation of mutarotation velocity with changes in pH.

EXPERIMENTS

Measurements of the optical rotations of sugar solutions were made by means of a Schmidt and Haensch polariscope which could be read to 0.01° . The polariscope tube was 400 mm. in length. It was provided with a jacket through which water was circulated from a large thermostat. The thermostat was maintained at 25.0°C. , but the temperature of the solutions was often slightly higher than this, due to absorption of heat from the surroundings. However, in any given run, the temperature was usually constant, and was determined by immersing a thermometer in the contents of the tube. The illumination was provided by a monochromator. Unfortunately, this instrument was out of adjustment, and the exact wave-length in use could not be ascertained. For this reason, the values found for the optical rotation of various solutions are not comparable with the data of others.

Even though the wave-length of the light was unknown, the fact that it remained constant during a series of experiments, made it possible to determine the velocity of mutarotation. It was also possible to determine the effect of various added substances upon the equilibrium rotation of lactose. Accordingly, the work reported in this, and in the following section, was limited to a study of these two phenomena.

If catalytic activity is a general property of proton donators and acceptors, then catalytic action might be expected from not only the hydrogen and hydroxyl ions, but also from the undissociated molecules of acids and bases, from the anions of weak acids, and the cations of weak bases.

It is well known that the velocity of mutarotation of lactose may be greatly altered by changes in the pH of its solutions. This effect will be discussed in more detail, later. At this time, it is sufficient to state that the velocity of mutarotation is practically constant through the pH range from three to six. In order that slight changes in pH might not obscure the effects of other catalysts, all experiments reported in this section were carried out at pHs between 4.0 and 5.0.

The velocity of mutarotation was determined by placing 5.00 grams of recrystallized alpha hydrate in a 100 cc. volumetric flask. This sugar was then dissolved in the solvent (water or salt solutions) and the flask was filled to the mark. After mixing thoroughly, the solution was transferred to the polariscope tube. A series of observations was then made at ten minute intervals from which the mutarotation velocity could be calculated. In order to increase the accuracy of these observations, each value was determined by making a series of readings in rapid succession, beginning shortly before the ten minute interval was ended. Later, by plotting these readings against time the most probable value of the rotation at the exact moment selected could be determined with considerable accuracy. The

rotation at equilibrium was determined after the solution had remained at approximately twenty-five degrees for twenty-four hours. The mutarotation constant, $k_1 + k_2$, was determined by means of the equation,

$$k_1 + k_2 = \frac{1}{t} \log \frac{V_0 - V}{V_t - V}$$

In this equation, V is the equilibrium rotation, V_t is the rotation at a time t hours after the initial observation, V_0 , was made. Logarithms to the base ten were used.

Using this method, the mutarotation velocity of lactose was determined in pure water, and in solutions of several salts. For these experiments, a normal solution of ammonium chloride was used to test the influence of a cation of a weak base, potassium acetate was used to test the influence of an anion of a weak acid, and ammonium acetate was used to test the combined influence of the two ions. As a control, the mutarotation of lactose was determined in pure water, and in solutions of potassium chloride, which was chosen as a representative of the salts of strong acids and strong bases. In each case, the solvent was acidified with HCl to a pH of approximately 5.0, as estimated by means of methyl red. The data are shown in Table 1.

TABLE 1
The mutarotation velocity of lactose in normal salt solutions

SALT USED	TEMPERATURE	$k_1 + k_2$
None	25.3° C.	0.475
Potassium chloride	25.0	0.413
Ammonium chloride	25.4	0.501
Potassium acetate	25.0	2.8
Ammonium acetate	25.0	3.5
Potassium chloride	25.0	0.404

All solutions were adjusted by means of hydrochloric acid and methyl red to a pH of approximately 5.0.

The results of these experiments indicate a marked catalysis of the mutarotation of lactose by the anions of weak acids. A normal solution of potassium acetate is more effective as a catalyst than a tenth normal solution of hydrogen ions, Table 2. On the other hand, though the anion of the weak base, ammonium hydroxide, exhibits a definite effect, it is very small compared with the effect of the acetate ion, even though the dissociation constants of acetic acid and of ammonium hydroxide are practically the same. This is in agreement with the fact that the hydrogen ion, a

TABLE 2
*Calculated values of the mutarotation constant of lactose at various values of pH.
 Temperature 25° C.*

pH	$k_1 + k_2$	pH	$k_1 + k_2$
0.0	19.5	5.0	0.46
0.5	4.7	6.0	0.48
1.0	1.74	6.5	0.54
1.5	0.94	7.0	0.68
2.0	0.65	7.5	1.04
2.5	0.52	8.0	2.07
3.0	0.48	8.5	5.9
4.0	0.46	9.0	27.6

These values are based on the data of Troy and Sharp (50).

proton donator, shows much less catalytic effect than the hydroxyl ion, a proton acceptor.

The action of potassium chloride seems anomalous, since it seems to retard the mutarotation of lactose. No explanation of this phenomenon has been found. At first, experimental error was suspected, but a repetition of the experiment with new reagents gave the same result. Finally, a search of the literature revealed that this phenomenon had been observed by others. Lowry (26) reported in 1903 that the mutarotation of glucose was retarded in normal solutions of potassium chloride. Mukhin and Ass (37) found, in 1925, that 0.5 normal potassium chloride reduced the mutarotation velocity of glucose by 6.1 per cent, and that 0.5 normal sodium chloride reduced it by 3.4 per cent. On the other hand, in 1903, Trey (49) reported that sodium chloride had no effect upon the mutarotation of glucose and Andrews and Worley (1) also reported that pure sodium chloride, in concentrations as high as 8.6 mols per thousand mols of water, had no effect.

If we accept the fact that, at high concentrations, KCl does retard the mutarotation of lactose, it becomes a matter of some interest as to whether this effect is due to the undissociated salt, to the potassium ion, or to the chloride ion. This matter has not yet been investigated.

As early as 1897, Trey (48, 49) reported that the mutarotation of lactose was accelerated by many salts. Similar effects have been observed by others with glucose. Trey believed that hydrolysis, with the consequent liberation of acid or of alkali, was responsible for this phenomenon. To what extent pH changes may account for his results cannot be determined at present. Mukhin and Ass believed that the phenomenon was due to the formation of compounds in solution which had a greater mutarotation velocity in solution than the free sugars. The results obtained in this investigation seem to support the theories of Lowry, and of Brönsted,

regarding acid and basic catalysis. However, it is quite possible that other factors might influence mutarotation velocities. Certainly a satisfactory theory must account for the retarded mutarotation of lactose in concentrated solutions of potassium chloride.

After having demonstrated that the mutarotation velocity of lactose was influenced by ions other than hydrogen and hydroxyl, it became desirable to determine the actual mutarotation velocity of lactose in milk, and in the various dairy products. Direct determination was not possible, however, because of the turbidity of such solutions. Until other means of determination could be developed, it was necessary to work with simple solutions. So far this part of the investigation has been limited to a study of the effect of lactates and lactic acid at various concentrations. Lactates were chosen as typical salts of weak acids. It is true that lactates are not found in sweet milk products, but other salts of weak acids are found there.

A lactate buffer solution was prepared from C.P. sodium carbonate and C.P. lactic acid. The solution had a pH of approximately 4.3. It was boiled for 15 minutes to expel all carbon dioxide. This solution contained one molecular weight of sodium lactate per liter, plus an excess of lactic acid. From this original solution, three solutions were prepared, by dilution, having one-tenth, one-hundredth, and one-thousandth the concentration of the original solution. Although the concentration of the undissociated acid, and of the lactate ion, in these solutions, varied through a range of 1 to 1000, the concentration of hydrogen ions was practically the same in each solution. Actually, the variation in hydrogen-ion concentration was only in the ratio of 1 to 2.3, and this variation is too small to cause any perceptible change in the velocity of mutarotation at the pH of these solutions.

The mutarotation velocity of lactose solutions (5 grams per 100 cc.) was determined in these four solutions which had practically the same pH, but different concentrations of salt and acid. The data which were obtained are recorded in Table 3.

It was necessary to make a small correction to the observed rotations of these solutions because the lactic acid used for the buffer was slightly dextro-rotatory. This correction, amounting to 1.12° in the case of the most concentrated buffer solution, was subtracted from all of the observations before the calculations of mutarotation velocity were made.

From the data in Table 3, it seems probable that the acid and salt in the most dilute buffer had no measurable influence upon the mutarotation velocity of the lactose. When the salt concentration was one-hundredth normal, the velocity increased slightly. At concentrations of tenth normal, the mutarotation constant was increased about 40 per cent. At higher concentrations, the influence of the salt and acid increased enor-

TABLE 3

The mutarotation constant of lactose in lactate buffer solutions of different concentrations. Temperature, 25° C.

CONCENTRATION OF SODIUM LACTATE	pH	$k_1 + k_2$		
		First trial	Second trial	Average
Normal	4.32	1.74	1.76	1.77
		1.79	1.78	
		1.74	1.84	
		(1.76)	(1.79)	
0.1 normal	4.32	0.625	0.675	0.636
		0.612	0.643	
		0.627		
		(0.621)	(0.659)	
0.01 normal	4.44	0.542	0.601	0.529
		0.511	0.487	
		0.517	0.515	
		(0.525)	(0.534)	
0.001 normal	4.68	0.500	0.451	0.472
		0.478	0.471	
		0.454	0.480	
		(0.477)	(0.467)	

mously. This is analogous to the effects of the hydrogen, or hydroxyl ions, whose catalytic power increases slowly at first, and then more rapidly as the concentration is increased.

If we attempt to apply these data to dairy products, it becomes apparent that lactic acid will seldom accelerate the mutarotation of lactose. Milk is coagulated by approximately 0.45 per cent of lactic acid. This is equivalent to only 0.05 normal, and at the resulting pH of $4.7 \pm$, a mutarotation constant of approximately 0.57 might be expected. This, however, is less than the value of the constant at a pH of 6.6–6.7 (the pH of fresh milk) in the absence of lactic acid.

At present, data regarding the effect of other proton donators and acceptors in milk are not available. However, if the catalytic action of other ions and molecules changes as rapidly with change of concentration as is true of lactates and lactic acid, then their action is probably of minor importance in unconcentrated products, but may be very important in such concentrated products as ice cream and milk powders. However, more data are necessary, particularly with respect to the catalytic action of proteins, before definite conclusions may be drawn.

Since molecules and ions other than hydrogen and hydroxyl influence the mutarotation velocity of lactose, it becomes necessary to examine the

data regarding the effect of pH upon this, to determine how greatly they might be in error due to interference by accelerating agents whose influence had not been recognized. The influence of acids and alkalies upon the mutarotation velocity of lactose has been shown by a number of workers (51, 52, 48, 49, 7, 50, 39). Of these, the last two have published curves showing the values of the mutarotation constant at various values of pH.

At first glance, the curves of Troy and Sharp (50) and those of Parisi (39) seem to differ considerably. Most of the difference, however, seems to arise from the fact that Parisi did not study the behavior of solutions of pH less than 1.6, and did not observe the marked acceleration of the mutarotation velocity which occurs below pH 1.0, a phenomenon known since 1882 (51).

We may examine the technique used in the two researches to estimate the likelihood of errors introduced by interfering catalysts. Parisi used unbuffered solutions. Troy and Sharp used very dilute buffers. The method of Parisi should be practically free from errors due to catalysts other than hydrogen and hydroxyl. The use of buffers is subject to criticism, but the buffer solutions used by Troy and Sharp were so dilute that the errors involved are practically negligible.

Parisi's paper contains some equations which were derived to show the relation between the values of k_2 , of temperature, and of pH. Others have attempted to write equations for the relation of $k_1 + k_2$ to the pH, but Parisi is the first to attempt to include temperature as one of the variables. In doing so, he introduced an error. In his derivation he substituted $K_w = 10^{-14}$, a substitution which is only valid at approximately 25° C. Because of variation in K_w (36), the constant 1.743×10^{12} of Parisi's equation should become 1.61×10^{12} at 40° C. and 1.79×10^{12} at 16° C.

Parisi's attempt to derive an equation relating the mutarotation constant to the pH suggested that the data of Troy and Sharp might serve as the basis for such an equation. An empirical equation was therefore derived which gives values agreeing very well with those determined by experiment. This equation may be written in the form,

$$\log (k_1 + k_2) = 0.00415 (\text{pH} - 4.45)^4 - 0.34$$

Using this equation, the values of the mutarotation constant were calculated for various values of pH. They are given in Table 2. These values are, of course, only applicable at temperatures of 25° C., and to solutions free from appreciable amounts of other catalytic substances.

SUMMARY

The mutarotation of lactose is accelerated by molecules, and by ions other than those of hydrogen and hydroxyl. This phenomenon is attributed to general acid and base catalysis.

The catalytic influence of the anions of weak acids is much greater than that of the cations of weak bases.

The catalytic effect of the lactate ion is relatively small at concentrations below one-tenth normal, but it increases very rapidly as the concentration becomes greater.

The catalytic effects of other salts found in dairy products have not yet been determined, but it seems probable that their influence would not be important, except possibly in such concentrated products as milk powder and ice cream.

An empirical equation, based upon the data of Troy and Sharp, has been derived for estimating the velocity of mutarotation of lactose solutions at 25° C., and at various values of pH.

REFERENCES

- (1) ANDREWS, J. C., AND WORLEY, F. P. Mutarotation. II. The relative velocities of mutarotation of alpha and beta glucose; effect of acid and salt. *J. Phys. Chem.* 31: 882-5. 1927.
- (2) ARMSTRONG, E. FRANKLAND. Studies on enzyme action. I. The correlation of the stereoisomeric alpha and beta glucosides with the corresponding glucoses. *J. Chem. Soc.* 83: 1305-13. 1903.
- (3) BAKER, J. W., INGOLD, C. K., AND THORPE, J. F. Ring chain tautomerism. IX. The mutarotation of the sugars. *J. Chem. Soc.* 125: 268-91. 1924.
- (4) BAKER, J. W. The mechanism of tautomeric interchange and the effect of structure on mobility and equilibrium. II. Ring chain tautomerism in its relation to the mutarotation of the sugars. *J. Chem. Soc.* 1583-93. 1928.
- (5) BAKER, J. W. The mechanics of tautomeric interchange and the effect of structure upon mobility and equilibrium. III. The function of alkaline and acid catalysts in the mutarotation of some derivatives of tetramethylglucose. *J. Chem. Soc.* 1979-87. 1928.
- (6) BAKER, J. W. The mechanism of tautomeric interchange and the effect of structure upon mobility and equilibrium. IV. Further evidence relating to the mechanism of acid catalysis in the mutarotation of nitrogen derivatives of tetraacetyl glucose. *J. Chem. Soc.* 1205-10. 1929.
- (7) BLEYER, B., AND SCHMIDT, H. Studien über das Verhalten der wichtigsten Kohlenhydrate in stark saur alkalischer sulfid und bisulfithaltiger Lösung. III. Einwirkung von Alkalien auf der Kohlenhydrate. *Biochem. Z.* 141: 278-96. 1923.
- (8) BRÖNSTED, J. N. The theory of acid and basic catalysis. *Trans. Faraday Soc.* 24: 630-40. 1928.
- (9) BRÖNSTED, J. N., AND GUGGENHEIM, E. A. Contribution to the theory of acid and basic catalysis. The mutarotation of glucose. *J. Am. Chem. Soc.* 49: 2554-84. 1927.
- (10) DUBRUNFAUT. Note sur le sucre de lait. *Compt. rend.* 42: 228-33. 1856.
- (11) ERDMANN, E. O. Dissertatio de saccharo lactico et amalaceo. Abstracted in *Jahresber. Chem.* 671-3. 1855.
- (12) ERDMANN, E. O. Über wasserfreien Milchzucker. *Ber.* 13: 2180-4. 1880.
- (13) FISCHER, E. Notizen über einige Säuren der Zucker-gruppe. *Ber.* 23: 2625-28. 1890.

- (14) GILLIS, J. Nouvelles recherches sur le sucre de lait. *Rec. trav. chim.* **39**: 88-125. 1920.
- (15) HALL, NORRIS F. Acid base equilibria in non aqueous solvents. *Chem. Reviews.* **8**: 191-212. 1931.
- (16) HUDSON, C. S. The forms of milk sugar. *Princeton University Bulletin* **13**: 62-6. 1902.
- (17) HUDSON, C. S. Über die Multirotation des Milchezuckers. *Z. physik. Chem.* **44**: 487-94. 1903.
- (18) HUDSON, C. S. The hydration of milk sugar in solution. *J. Am. Chem. Soc.* **26**: 1065-82. 1904.
- (19) HUDSON, C. S. The catalysis by acids and bases of the mutarotation of glucose. *J. Am. Chem. Soc.* **29**: 1571-6. 1907.
- (20) HUDSON, C. S. Further studies on milk sugar. *J. Am. Chem. Soc.* **30**: 1767-83. 1908.
- (21) HUDSON, C. S. A review of discoveries on the mutarotation of sugars. *J. Am. Chem. Soc.* **32**: 889-94. 1910.
- (22) HUDSON, C. S., AND BROWN, F. C. The heats of solution of the three forms of milk sugar. *J. Am. Chem. Soc.* **30**: 960-71. 1908.
- (23) JONES, G. G., AND LOWRY, T. M. Dynamic isomerism. XXI. Velocity of mutarotation of tetra methyl glucose and of tetra acetyl glucose in aqueous acetone. *J. Chem. Soc.* 1926: 720-3. 1926.
- (24) KILPATRICK, M., JR., AND KILPATRICK, MARY L. Acid and basic catalysis. *Chem. Reviews* **10**: 213-27. 1932.
- (25) LOWRY, T. M. Study of the terpenes and allied compounds, nitrocamphor and its derivatives. IV. Nitrocamphor as an example of dynamic isomerism. *J. Chem. Soc.* **75**: 211-44. 1899.
- (26) LOWRY, T. M. Studies of dynamic isomerism. I. The mutarotation of glucose. *J. Chem. Soc.* **83**: 1314-23. 1903.
- (27) LOWRY, T. M. Studies of dynamic isomerism. III. Solubility as a means of determining the proportions of dynamic isomerides in solution. Equilibrium in solutions of glucose and galactose. *J. Chem. Soc.* **85**: 1551-70. 1904.
- (28) LOWRY, T. M. Dynamic isomerism. XVIII. Mechanism of mutarotation. *J. Chem. Soc.* **127**: 1371-85. 1925.
- (29) LOWRY, T. M. Dynamic isomerism of the reducing series. *Z. physik. Chem.* **130**: 125-45 (in English). 1927.
- (30) LOWRY, T. M. Studies in dynamic isomerism. XXV. The mechanism of catalysis by acids and bases. *J. Chem. Soc.* (1927): 2554-65. 1927.
- (31) LOWRY, T. M. Static and dynamic isomerism in prototropic compounds. *Chem. Reviews* **4**: 231-53. 1928.
- (32) LOWRY, T. M., AND FAULKNER, I. J. Studies in dynamic isomerism. XX. Amphoteric solvents as catalysts for the mutarotation of sugars. *J. Chem. Soc.* **127**: 2883-7. 1925.
- (33) LOWRY, T. M., AND WILSON, GORDON L. Determination of the catalytic coefficient of the hydroxyl ion in the mutarotation of glucose and galactose. *Trans. Faraday Soc.* **24**: 683-7. 1928.
- (34) LOWRY, T. M., AND RICHARDS, E. M. Experiments on the arrested mutarotation of tetra methyl glucose. *J. Chem. Soc.* **127**: 1385-401. 1925.
- (35) LOWRY, T. M., AND SMITH, G. F. Studies in dynamic isomerism. XXIX. Neutral salt action in mutarotation. *J. Chem. Soc.* (1927): 2539-54. 1927.
- (36) MICHAELIS, LEONOR. Hydrogen Ion Concentration (Translated from 2nd edition). Williams and Wilkins, Baltimore. 1926.

- (37) MUKHIN, G., AND ASS, T. Action of salts on the mutarotation of glucose. *J. chim. Ukraine*. 1: 458-75 (1925) (not seen). *Chem. Zentr.* (1926) 1: 620 (1926). *C. A.*, 20: 2775. 1926.
- (38) OSAKA, YUKICHI. Über die Birotation der d-Glukose. *Z. physik. Chem.* 35: 661-706. 1900.
- (39) PARISI, PERICLE. L'influenza della "reazione" nella tecnica di preparazione del lattosio, del latte condensato e della crema gelata. *Giorn. chim. ind. applicata*. 12: 225-35. 1930.
- (40) RICHARDS, E. M., FAULKNER, I. J., AND LOWRY, T. M. Dynamism isomerism. XXII. Mutarotation in aqueous alcohols. *J. Chem. Soc.* (1927): 1733-9. 1927.
- (41) ROUX, E. Sur la polyrotation des sucres. *Ann. chim. phys.* (7) 30: 422-32. 1903.
- (42) SCHMOEGER, M. Eine bis jetzt noch nicht beobachtete Eigenschaft des Milchezuckers. *Ber.* 13: 1915-21. 1880.
- (43) SCHMOEGER, M. Nachtrag zu meiner Mittheilung: "Eine bis jetzt noch nicht beobachtete Eigenschaft des Milchezucker," in diesen Berichten. *Ber.* 13: 2130-2. 1880.
- (44) SCHULTZE, C., AND TOLLENS, B. Ueber das Verschwinden der Multirotation der Zuckerarten in ammoniakalischer Lösungen. *Ann.* 271: 49-54. 1892.
- (45) TANRET, C. Sur les modifications moleculaires du glucose. *Bull. soc. chim.* (3) 13: 728-35. 1895.
- (46) TANRET, C. Sur les modifications moleculaires et la multirotation des sucres. *Bull. soc. chim.* (3) 15: 195-205. 1896.
- (47) TREY, HEINRICH. Experimentalbeitrag zur Birotation der Glykose. *Z. physik. Chem.* 18: 193-218. 1895.
- (48) TREY, HEINRICH. Ein weiter Beitrag zur Birotation der Glycose. *Z. physik. Chem.* 22: 424-63. 1897.
- (49) TREY, HEINRICH. Ein Beitrag zu den Rotationserscheinungen der Laktose. *Z. physik. Chem.* 46: 620-719. 1903.
- (50) TROY, H. C., AND SHARP, PAUL FRANCIS. Alpha and beta lactose in some milk products. *JOURNAL DAIRY SCIENCE* 13: 140-57. 1930.
- (51) URECH, F. Zur strobometrischen Bestimmung der Invertirungsgeschwindigkeit von Rohrzucker und des Uebergangs von Milchezucker zu seiner constanten Drehung. *Ber.* 15: 2130-33. 1882.
- (52) URECH, F. Ursachlicher Zusammenhang zwischen Löslichkeits und optischer Drehungserscheinungen bei Milchezucker und Formulirung der Uebergangsgeschwindigkeit seiner Birotation in die Normale. *Ber.* 16: 2270-71. 1883.

LESPEDEZA STRAW FOR DAIRY COWS

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The palatability and value for milk production of lespedeza straw were studied in a feeding trial with dairy cows. Comparison was made with soybean hay, chosen because of the fact that both lespedeza and soybeans, unlike most other legume crops used in feeding dairy cattle, can be grown on acid soils.

A number of studies of the feeding value of lespedeza hay have been made. Moore (1) reports "very little difference in the feed value of these legumes (alfalfa, lespedeza and soybean hays) when all are cut at the right state and properly cured."

Moore and Cowser (2) reported that dairy cows produced slightly less milk but gained more weight when fed lespedeza hay than when soybean hay was fed. In a comparison (2) of lespedeza and alfalfa hays for milk production, it was found that less feed was required to produce a unit of product when alfalfa was given.

The North Carolina Station (3) reported a slight advantage of alfalfa hay over lespedeza hay for dairy cattle.

The Tennessee Station (4) reported that *Sericea lespedeza* hay, carrying about 25 per cent of crab grass, gave better results with yearling Jersey heifers than grass hay, but the gains were not equal to those made with alfalfa.

The author has been unable to locate in the literature any reports of experimental investigations of the feeding value of lespedeza straw for dairy cattle.

DESCRIPTION OF FEEDS USED IN TRIAL

The *lespedeza straw* came from Korean lespedeza grown for seed in Crawford County, Illinois. The crop was harvested before complete maturity of the seed and was threshed and baled without exposure to rain. The leaves were saved carefully. Approximately 65 per cent of the straw consisted of leaves, 27 per cent of stems and 8 per cent of foreign matter composed principally of timothy stubble. At the time of feeding, part of the leaves were green and part light brown in color. The stems were dark brown or brownish red. The hay was free from mold and mustiness and was in good condition for feeding. The stems were hard and somewhat wiry.

The *soybean hay* was grown on the University Farm and was harvested when the pods were forming. The pods were almost devoid of seed. The

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hay was cured without exposure to rain, baled in the field and stored at once. The hay was bright green in color and was free from mold, musty odors and excessive dust. Portions of several bales were taken during the feeding trial and the sample thus obtained separated into pod, stem, and leaf (entire leaf including petiole) portions. The pods comprised about 5 per cent, the stems 31 per cent, and the leaves 64 per cent of the sample.

The *corn silage* was of good quality. It was made from heavily-eared Reid Yellow Dent Corn, well advanced in maturity. It contained about 32 per cent dry matter.

The *grain mixture* was made up according to the following formula: shelled corn, ground, 425 pounds; oats, ground, 200 pounds; wheat bran, 250 pounds; soybean oil meal, 100 pounds; salt, 15 pounds; steamed bone meal, 10 pounds. It contained approximately 14 per cent total protein.

GENERAL PLAN OF FEEDING TRIAL

The trial consisted of two periods, each four weeks in length. Period I was preceded by a 2-weeks' preliminary period and Period II by a 1-week transition period.

Two groups of cows were used. Each group consisted of eight Holsteins and one Ayrshire. The groups were made up by choosing two cows as nearly alike as possible with respect to stage of lactation, milk yield, etc., and placing one cow in Group I and the other in Group II.

The Holsteins were fed 30 pounds of silage per head daily, and the Ayrshires 20 pounds. Toward the close of the trial, it was necessary to reduce the amounts fed to some of the cows on account of a higher acid condition in the silage as the bottom of the silo was neared.

The hay and straw were fed in amounts such that each group consumed approximately the same number of pounds. The soybean hay, however, was limited to amounts which would be eaten without waste of leaves, pods and fine portions of the stems. Only the coarse stems remained. It was not possible to adjust the feeding of lespedeza straw so closely, because the cows did not make a clean separation of the leaves from the stems. The refused portions of the hay and straw were collected from the mangers frequently and placed in large gunny sacks. Weekly records were made of the refusal of each cow. The refused portions, which absorbed moisture as a result of being nosed over by the cows, were dried and the amounts calculated to the same moisture basis as the hay fed. Samples of all feeds for analysis were taken regularly.

At the close of Period I, the group of cows which had been fed lespedeza straw was fed soybean hay and the group which had received soybean hay during Period I was given lespedeza straw.

Each milking of each cow was weighed and sampled. Composite samples were tested weekly for butterfat content. The cows were weighed on

three consecutive days at the beginning and end of each period and also once weekly.

COMPARATIVE FEEDING VALUES

Lespedeza straw was not as palatable as the soybean hay. There was, however, considerable variation in the preferences of the cows. Eight of the cows consumed as much of the lespedeza straw as of the soybean hay.

A true picture of the relative palatability of the two feeds is not given by the figures shown in Tables 1 and 2, because the amounts of soybean hay fed to a number of cows were limited in order to keep the dry roughage con-

TABLE 1
Summary of results of feeding lespedeza straw in comparison with soybean hay

KIND OF FEED	NO. OF COWS	FEED CONSUMED DAILY PER COW			GAIN IN WEIGHT DAILY PER COW	TEST OF MILK	MILK YIELD DAILY PER COW ¹
		Silage	Straw or hay	Grain			
		<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>	%	<i>lbs.</i>
Lespedeza straw	18	28.4	12.0	12.8	.37	3.71	33.5
Soybean hay	18	28.4	12.8	12.8	.08	3.78	35.5

¹ Milk energy in terms of 4% milk computed according to the formula $.4 \times \text{milk (in pounds)} + 15 \times \text{fat (in pounds)}$.

TABLE 2
Proportions of straw and hay refused

KIND OF FEED	NO. OF COWS	AMOUNT FED DAILY PER COW	AMOUNT REFUSED DAILY PER COW		AMOUNT CONSUMED DAILY PER COW
		<i>lbs.</i>	<i>lbs.</i>	%	<i>lbs.</i>
Lespedeza straw	18	13.3	1.3	10.0	12.0
Soybean hay	18	16.6	3.8	23.1	12.8

sumption of the two groups of cows approximately the same. Probably more soybean hay would have been consumed had it been offered, but this would not have been likely for the lespedeza straw except in the case of one cow, because all cows in the lespedeza group were fed to the limit of appetite except for this one cow.

Milk production was slightly less while the cows were fed lespedeza straw than when they received soybean hay, but gains in weight were slightly greater when the cows were fed the lespedeza (Table 1). Ten per cent of the lespedeza straw fed was refused while 23 per cent of the soybean hay was refused (Table 2).

The lespedeza straw was much lower in protein and somewhat higher in fiber content than the soybean hay (Table 3). The refused portions of the

TABLE 3
Composition of lespedeza straw and soybean hay

KIND OF ROUGHAGE	DRY MATTER	ASH	TOTAL PROTEIN	FIBER	ETHER EXTRACT	N-FREE EXTRACT
	%	%	%	%	%	%
Soybean hay	90	8.0	12.6	25.9	1.5	42.0
Lespedeza straw	90	4.6	6.8	29.2	2.3	47.1
Soybean stems ¹	90	3.5	7.7	43.1	.9	34.8
Lespedeza stems ¹	90	3.6	5.1	36.6	1.3	43.4

¹ Portions of roughage refused by cows during feeding trial.

straw and hay were lower in protein and higher in fiber than the feeds from which they came, indicating that the refuse consisted of the coarser portions of the feeds.

SUMMARY

Lespedeza straw consisting of the roughage from a crop of lespedeza harvested before maturity and threshed for seed, proved somewhat less palatable and slightly less valuable as a feed for dairy cows in milk than soybean hay harvested in the early stages of pod formation.

Twenty-three per cent of the soybean hay fed was refused while only ten per cent of the lespedeza straw was refused.

REFERENCES

- (1) MOORE, J. S., Miss. Agr. Exp. Sta., Rpt., p. 17. 1925.
- (2) MOORE, J. S., AND COWSERT, W. C., Soybeans for Dairy Cows, Miss. Agr. Exp. Sta., Bul. 235. 1926.
- (3) N. Car. Agr. Exp. Sta., Rpt., p. 53. 1932.
- (4) Tenn. Agr. Exp. Sta., Rpt., p. 60. 1931.

FAT RISING IN CREAM¹

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A study of the extent to which butterfat tends to rise in cream was undertaken primarily to seek an explanation for the distribution of butterfat in frozen cream. Moreover, the dearth of literature pertaining to the rise of butterfat in cream required that a more complete study be made in order to understand better the effect of the factors involved.

The abundance of literature on the rise of butterfat in milk offers a good fund of information concerning the phenomenon of fat rising. The fundamental cause of cream rising is the difference between the specific gravity of butterfat and the plasma in which the fat globules are dispersed. Various investigators have attributed the differences in the rapidity and completeness with which the fat rises in milk to the size of the fat globules and the degree of their association, the plasma-solids content of the milk, heat treatments to which milk is subjected, viscosity, individuality of the animal producing the fat, electric charges on the fat globules, and agitation of the milk.

Evidence which gave definite data concerning the distribution of fat in cream was found in the paper reported by McInerney and Sharp (4) on fat distribution in gravity cream. Their experimental results showed that the fat content of the cream layer in milk bottles decreased progressively from the top to the bottom. Fundamental evidence supporting this work was presented by Troy and Sharp (9) in their rather complete study of the physical factors involved in fat rising of milk. Dahlberg and Marquardt (1) have also secured analyses of fractional parts of gravity cream which indicate that the lower fraction tests less than the top portion. A progressive decrease in the fat content of the skimmilk below the cream line was found by McInerney and Sharp, and Dahlberg and Marquardt. However, these studies were made on gravity creams which were produced under entirely different conditions than those separated centrifugally. While these investigators demonstrated the possibility of a range in the fat content from the top to the bottom of cream, this cannot be taken as evidence that such is always the case, particularly with mechanically separated creams containing higher percentages of butterfat.

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EXPERIMENTAL METHODS

Three rather distinct types of cream were used for these studies, *i.e.*, low fat cream containing near 20 per cent butterfat, medium fat cream with about 30 per cent butterfat and high testing cream containing approximately 40 per cent fat.

Methods somewhat similar to those employed by Palmer and Anderson (6) in their estimations of cream rising in milk were used for this experiment. Cream was placed in 200 milliliter graduated cylinders, in the bottom of which holes $3/16$ inch in diameter had been bored so that fractional parts of the cream could be drawn off separately with a minimum amount of mixing taking place. Small wooden plugs were used to stop the holes until such time as the holding period was over when the stopper was removed and the cream permitted to flow out slowly.

Samples from the lower, middle and top fractions of the cream were drawn off separately after the cylinder had been permitted to stand the required length of time. The sample representing the lower fraction was the bottom 20 milliliters and the top fraction consisted of the last 20 milliliters taken out of the cylinder, it being the upper tenth portion of the cream. These samples were kept separate and tested individually so that a comparison of the tests could be used to ascertain the degree of fat rising which had occurred in the cream that was held in the cylinder.

For each cream study made, holding periods of 6, 12, 18, and 24 hours were maintained. This was accomplished by mixing the cream thoroughly immediately after it was standardized to insure a uniform distribution of the fat. Five cylinders were then filled with the mixed cream. The cylinders were all placed on a rack which was put into a cooler where it would not be disturbed. At the end of six hours the first cylinder was removed from the rack and fractionated. The second cylinder was removed after 12 hours, the third after 18 hours, and so on. In case any of the first four cylinders were not correctly sampled, the fifth cylinder was used in its stead, but when no accidents occurred, the fifth cylinder was used for observing the progress of the fat rising after a longer holding period.

Raw Cream

Six trials were conducted with raw cream which represented the three different fat contents. In some cases the cream was standardized to the required fat percentage. The cream was cooled to 40° F. before it was placed in the cylinders and the temperature of the cooler was maintained below that temperature and above freezing at all times. The data presented in table 1 represent the average results for the six trials.

The average figures show that the distribution of fat in high-testing creams was fairly uniform even after they had been allowed to stand for 24 hours. Nevertheless, a slow gradual rise of the butterfat appeared to

TABLE 1

Average butterfat tests of top, middle, and bottom fractions of raw cream held below 40 degrees F.

TYPE OF CREAM	FRACTION	BUTTERFAT CONTENT AFTER			
		6 hours	12 hours	18 hours	24 hours
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
High fat	Top	39.58	39.71	39.75	40.17
	Middle	39.42	39.50	39.58	39.58
	Bottom	39.42	39.21	39.37	39.17
Medium fat .	Top	30.21	30.71	31.33	31.42
	Middle	30.21	30.27	30.21	30.21
	Bottom	30.33	30.08	29.92	29.81
Low fat	Top	20.92	21.75	24.50	23.75
	Middle	20.10	20.14	20.25	20.33
	Bottom	18.17	17.00	15.67	15.00

take place, which rise, at the end of a day, caused the top portion to test almost one per cent higher than the bottom portion. Although these are average results, in no case did the top sample test less than 0.5 per cent more than the bottom fraction. The maximum difference between the top and bottom fractions was 1.5 per cent of fat. No greater differences were found when a two-day holding period was used. The fat rise in this type of cream proved to be slow but definite at temperatures below 40° F.

In the cream of medium fat content the fat rise was somewhat greater inasmuch as the differences between the top and bottom fractions were on the average about twice as large as in the high fat creams. When this type of cream was permitted to stand for periods longer than one day, there was a continued slow rising of the fat.

The low-fat cream was subject to far more fat rising than the other two types. The average results show that in the course of six hours the fat rose to a greater extent than it did in the medium fat cream during 24 hours. At the end of one day the difference in tests between the top and bottom fractions was at least 6.5 per cent and the average for all the trials was more than 8 per cent. The rise of the fat in this type of cream continued to be very marked beyond 24 hours and in one sample held for 168 hours the top portion tested 34 per cent while the bottom portion contained less than 9.5 per cent fat.

In every trial the test of the middle portion remained practically the same as the original test of the cream. The loss of fat to the upper part was probably compensated by the gain from the lower portion. However, it was particularly noticeable in the low fat creams that the fat was more likely to leave the bottom fraction than it was to concentrate in the top por-

tion. This resulted in a tendency for a middle portion to test slightly more as the holding periods became longer. The increase in the fat content of the middle portion was evidently small because this fraction was eight times as large as the other fractions.

Holding Temperatures

Single trials were conducted with the three types of cream held at higher temperatures in order to demonstrate the influence that holding temperatures may exert on the fat rising. In one case, the creams were placed in a room maintained at 54° F.; and in the other, the creams were kept in a room at prevailing summer temperatures, namely, for the high fat cream, 84° F.; for the medium fat cream, 82° F.; and for the low testing cream, 76° F. There was a maximum variation of 1° F. in these holding temperatures. One per cent of formalin was added to the cream to prevent

TABLE 2
Butterfat content of top, middle, and bottom fractions of raw cream held at 54° F. and at room temperature

TYPE OF CREAM	FRACTION	BUTTERFAT CONTENT AFTER			
		6 hours	12 hours	18 hours	24 hours
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
		Held at 54° F.			
High fat	Top	39.0	38.5	39.5	40.0
	Middle	38.5	38.5	38.5	38.5
	Bottom	38.5	38.5	38.0	38.0
Medium fat	Top	29.0	30.0	31.5	32.0
	Middle	29.0	29.0	29.0	29.0
	Bottom	28.0	27.0	25.5	26.5
Low fat	Top	21.0	22.5	25.5	26.0
	Middle	20.5	20.5	20.5	20.5
	Bottom	18.0	16.0	14.0	14.5
		Held at Room Temperatures			
High fat	Top	41.5	41.0	42.0	42.0
	Middle	39.5	39.5	39.5	39.5
	Bottom		39.5	39.5	39.0
Medium fat	Top	33.5	35.0	36.0	36.0
	Middle	31.0	30.7	30.6	30.7
	Bottom	30.5	30.0	27.5	25.0
Low fat	Top	25.2	1	31.0	33.0
	Middle	20.6		20.5	20.5
	Bottom	17.5		10.0	7.0

• Samples discarded.

souring and curdling which would form a mechanical obstruction, thus preventing fat from rising.

The results of these trials, presented in table 2, indicate that holding temperatures have a decided influence upon the extent to which fat rises in cream. The most pronounced differences were evident in the low testing cream, the bottom tenth of which contained only 7 per cent of butterfat after holding for 24 hours at room temperature. The high fat cream was least affected, nevertheless, the influence was decided, as there was a three per cent difference between the top and bottom portions at the high temperature. In fact the difference between the tests of the top and bottom samples was approximately three times greater at room temperature than at 40° F. in all three types of cream.

Obviously, these holding temperatures were distinctly higher than would be expected in practice for such long periods of time. Nevertheless, the tendency of high temperatures to aid fat rising, and low temperatures to hinder it, was illustrated.

Pasteurized Creams

Cream of all three types was pasteurized at 150° F. for 30 minutes after which it was cooled and placed in the graduated cylinders. Four times this was tried with each type and the cylinders were held below 40° F. The average results of these trials appear in table 3.

TABLE 3

Average butterfat content of top, middle, and bottom portions of pasteurized creams held below 40° F.

TYPE OF CREAM	FRACTION	BUTTERFAT CONTENT AFTER			
		6 hours	12 hours	18 hours	24 hours
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
High fat	Top	41.50	41.38	41.87	42.00
	Middle	41.06	41.00	41.00	41.06
	Bottom	40.75	40.75	40.62	40.75
Medium fat	Top	30.12	30.37	31.37	31.88
	Middle	29.69	29.81	29.87	29.62
	Bottom	29.00	28.75	28.12	28.31
Low fat	Top	21.83	23.33	25.83	26.00
	Middle	20.87	21.25	21.33	21.50
	Bottom	16.00	14.33	12.83	12.50

Using the average figures of the raw cream trials as the basis for comparison, the data for pasteurized cream show the same general trend in the results obtained, but it is apparent that the fat tends to rise to a greater extent in the pasteurized cream. It is interesting to note that one sample of

medium fat cream was held for a period of three weeks, after which the top fraction tested 49.5 per cent, the bottom fraction less than one per cent and the test of the middle portion increased one per cent.

Pasteurized Milk

Palmer, Hening, and Anderson (6) secured results indicating that the effects of heat on cream rising were due to the effects of heat on the milk plasma. Therefore, it was planned to pasteurize a quantity of milk and separate the cream to note if there were any differences in the fat rising which took place in the resulting cream.

Eight gallons of milk obtained from the University herd were pasteurized in a hot water bath at 150° F. for 30 minutes, then cooled at 90° F. and separated. A cream of medium fat content was secured and cooled at 40° F. for the fat rising study. The cylinders were filled and held in a cooler maintained at 35° F. The results are presented in table 4.

TABLE 4

Butterfat content of top, middle, and bottom portions of cream from pasteurized milk

FRACTION	BUTTERFAT CONTENT AFTER				
	6 hours	12 hours	18 hours	24 hours	74 hours
Top	<i>per cent fat</i> 30.5	<i>per cent fat</i> 30.5	<i>per cent fat</i> 31.0	<i>per cent fat</i> 31.25	<i>per cent fat</i> 32.25
Middle	30.5	30.5	29.75	30.25	30.50
Bottom	30.5	30.5	30.5	30.50	27.75

Judging from these data, the fat rising seems to be delayed in cream separated from pasteurized milk for there was no apparent rise until after 12 hours of holding. At the end of one day there was less difference between the top and bottom tests of this cream than there was in the other pasteurized cream trials.

Standardization

The effect of standardization was studied by separating one batch of cream to approximately 35 per cent butterfat and another to 46 per cent. The 46 per cent cream was then standardized with skim-milk to nearly the same fat content. These creams were placed in the graduated cylinders and held at 35° F. The results will be found in table 5.

These data would not lead one to expect any marked rising of the fat in cream because of standardization. There was evidence of the fat moving upward somewhat faster at the beginning in the standardized cream, but at the end of 24 hours the differences between the top and bottom tests were practically the same. The results indicate that standardization is

TABLE 5

Butterfat content of various portions of standardized and unstandardized creams

TYPE OF CREAM	FRACTION	BUTTERFAT CONTENT AFTER			
		6 hours	12 hours	18 hours	24 hours
Standardized	Top	<i>per cent fat</i> 36.0	<i>per cent fat</i> 36.0	<i>per cent fat</i> 36.25	<i>per cent fat</i> 36.0
	Middle	35.5	35.5	35.5	35.5
	Bottom	35.5	35.5	35.5	34.5
Not Standardized	Top	34.5	35.25	35.5	36.0
	Middle	34.5	34.75	34.5	34.5
	Bottom	34.5	34.5	34.5	34.0

almost a negligible factor in influencing the distribution of butterfat in cream.

Plasma Separation

The recent studies concerning the separation of plasma (usually referred to as "serum" or "milk layer") from bottled cream are more intimately connected with the problem of fat rising in cream. Trout and McCann (8), Garrison and Powell (3), and Mooney and Burgwald (5) have contributed to the knowledge concerning this problem. In addition, Doan (2) has made recommendations for reducing or controlling this separation. There was a general agreement among these investigators that high pasteurization temperatures reduce the plasma layer; that the less standardization required, the less plasma separation; that long storage periods favor the separation; and that high storage or creaming temperatures also favor the separation, although the distinctness of the plasma layer is decreased. However, there was a difference of opinion and in results obtained in regard to the effects of separation temperatures, agitation, heat treatment of the medium for standardization, and the time for standardizing.

During the preliminary trials for the fat rising studies, it was noticed that the cream upon standing formed a plasma layer at the bottom of the graduated cylinders. It was further noted that the plasma layer was not uniform or consistent in quantity or distinctness from one type of cream to another. Doubtless this observation has been made in practical creamery operation for whipping cream seldom shows a noticeable plasma layer. On the other hand it is frequently encountered in coffee creams and is considered quite objectionable. This accounts for the fact that the above investigators have all confined their studies to low fat creams. Inasmuch as it was convenient to make observations concerning this plasma layer, it was decided to estimate the quantity formed as closely as possible and record the results. These observations were made when the cylinders were

TABLE 6

Plasma separation occurring in creams held under different conditions

TYPE OF CREAM	SAMPLE NO.	EXTENT OF PLASMA SEPARATION AFTER			
		6 hrs.	12 hrs.	18 hrs.	24 hrs.
		ml.	ml.	ml.	ml.
<i>High Fat Cream</i>					
Raw held below 40° F.	1	+*	+	+	+
	2	0	+	0.25	0.5
	3	+	+	++	++
	4	0	0	+	+
	5	0	0	0	+
Pasteurized—held below 40° F.	1	+	+	++	++
	2	0	0	0	+
	3	0	0	+	+
	4	+	+	+	+
Raw—held at 54° F.	1	+	+	+	+
held at 84° F.	1	+	0.5	1.0	1.0
<i>Medium Fat Cream</i>					
Raw—held below 40° F.	1	0	0.25	0.5	1.0
	2	0	0.25	0.75	1.0
	3	0	0.25	0.75	1.0
	4	0	+	+	0.5
held at 54° F.	1	+	+	+	0.5
held at 83° F.	1	+	1.0	2.0	3.0
Pasteurized—held below 40° F.	1	+	0.25	0.5	0.75
	2	+	+	0.5	++
	3	0	+	1.0	1.0
Pasteurized milk—held below 40° F.	1	0	0	0	+
<i>Low Fat Cream</i>					
Raw—held below 40° F.	1	0.5	1.0	2.0	2.5
	2	0.5	1.0	1.75	2.0
	3	0.5	2.0	2.0	2.5
	4	1.0	1.5	2.0	2.0
	5	1.0	2.0	4.0	4.0
	6	+	1.0	1.5	2.0
held at 54° F.	1	0.5	1.0	4.0	3.5
held at 65° F.	1	2.0	2.0	4.0	5.0
Pasteurized—held below 40° F.	1	0.5	++	2.0	2.0
	2	+	++	2.0	2.0
	3	+	0.25	1.5	1.75
35% standardized raw held below 40° F.	1	+	+	+	0.5

-trace.

-less than .25 milliliters.

removed from the rack for the purpose of draining the cream. Table 6 includes the observations made on the plasma separation.

Of the factors studied, the fat content of the cream seemed to exert the greatest influence on the plasma separation. The separation in high fat cream was almost negligible, but the low fat cream always exhibited a distinct plasma layer after standing. The medium testing cream produced a plasma layer that was intermediate in depth between that in the high and low fat cream.

High holding temperatures increased the plasma layer to a very marked degree with all three types of cream. A standardization study was carried out only in one trial and the results did not deviate notably from the results that might have been expected with the other raw creams.

Pasteurization of the cream appeared to have a tendency to reduce the plasma separation slightly. The line of demarkation of the plasma layer was less distinct in the pasteurized cream. When the milk was pasteurized, and medium-fat cream separated from it, less plasma separation was evident.

As the length of the holding period increased, the plasma layer invariably increased in size. In the sample of medium-fat pasteurized cream held for three weeks the plasma layer was 22 milliliters and did not contain enough fat to be read in a cream test bottle. It suffices to say, that in general the observations on the plasma formation made in this experiment corroborate the results secured by the investigators mentioned previously.

SUMMARY AND CONCLUSIONS

1. The butterfat in cream has a tendency to rise and concentrate in the upper portion. This phenomenon is somewhat similar to cream rising in milk.
2. Fat rising in cream is decreased as the butterfat content is increased.
3. Low holding temperatures reduce the amount and rapidity of fat rising in cream.
4. Pasteurization of cream appears to increase slightly the extent of fat rising.
5. Standardization of cream with skimmilk does not appreciably affect the amount of fat rising.
6. Low fat contents and high holding temperatures tend to increase plasma separation in cream.

REFERENCES

- (1) DAHLBERG, A. C., AND MARQUARDT, J. C. The creaming of raw and pasteurized milk. N. Y. State (Geneva) Agr. Expt. Sta. Tech. Bul. 157: 46-55, 74. 1929.
- (2) DOAN, F. J. Coffee cream wheys off. Natl. Butter and Cheese Jour. 24: No. 6, p. 16. 1933.

- (3) GARRISON, E. R., AND POWELL, M. E. Methods of reducing and preventing serum separation in twenty per cent cream without homogenization. Mo. Agr. Expt. Sta. Bul. 310: 29-30. 1932.
- (4) MCINERNEY, T. J., AND SHARP, P. F. The distribution of fat in gravity cream. Internatl. Assoc. Dairy and Milk Insp. Ann. Rept. 17: 256-260. 1928.
- (5) MOONEY, J. L., AND BURGWARD, L. H. Factors influencing the formation of milk layer in bottle coffee cream. Amer. Dairy Sci. Rpt. Ann. Meeting, Lexington, Ky. 1932.
- (6) PALMER, L. S., AND ANDERSON, E. O. Physico-chemical factors influencing cream rising. I. Viscosity. JOURNAL OF DAIRY SCIENCE 9: 1-14. 1926.
- (7) PALMER, L. S., HENING, J. C., AND ANDERSON, E. O. Physico-chemical factors influencing cream rising. II. Relation of plasma colloids to pasteurization effects. JOURNAL OF DAIRY SCIENCE 9: 171-191. 1926.
- (8) TROUT, G. M., AND McCANN, J. C. A study of several factors in the separation of serum from bottled cream. Jour. Agr. Res. 45: 483-500. 1932.
- (9) TROY, H. C., AND SHARP, P. F. Physical factors influencing the formation and fat content of gravity cream. JOURNAL OF DAIRY SCIENCE 11: 189-229. 1928.

THE RELATIVE EFFICIENCIES OF IRRADIATED ERGOSTEROL AND IRRADIATED YEAST FOR THE PRODUCTION OF VITAMIN-D MILK¹

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It has been known for some time that the effectiveness of vitamin-D supplements depends upon the nature of the vitamin carrier. Hess, Weinstock, and Rivkin (1) showed that fewer rat units of vitamin D in the form of cod liver oil than in the form of viosterol were needed to protect infants against rickets. Hess and Lewis extended this observation to different kinds of vitamin-D milks which led to a differentiation between the rat unit value of an anti-rachitic and its clinical unit value (2). Numerous investigators (3) (4) (5) (6) (7) (8) have shown that the chick requires several times more rat units of vitamin D in the form of irradiated ergosterol than in the form of cod liver oil. Hess, Lewis, MacLeod, and Thomas (9) found this difference in effectiveness to apply to cows, 200,000 rat units of vitamin D in the form of irradiated ergosterol having been required per cow daily to produce milk having the same vitamin-D potency as that produced by cows fed 60,000 rat units daily in the form of irradiated yeast. Krauss, Bethke, and Monroe (10) further demonstrated that when irradiated ergosterol is fed to cows the vitamin D in the butterfat produced by these cows does not act in the same order of magnitude as does an equivalent amount of vitamin D in cod liver oil when fed to chicks.

Since irradiated ergosterol was at the time still being seriously considered as a means by which the vitamin-D content of milk might be increased through feeding, it was felt desirable to obtain further information as to the relative efficiencies of irradiated ergosterol and irradiated yeast for this purpose.

EXPERIMENTAL

Four groups of three Holstein cows each were fed according to the schedule in Table 1. In Experiment I the basal ration consisted of alfalfa hay of excellent quality, dried beet pulp, corn, oats, bran, and linseed oil-meal. In the second experiment alfalfa hay and corn silage furnished the

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roughage part of the ration while the grain mixture remained the same as in experiment 1. Different cows were used in each experiment.

TABLE 1
Feeding Schedule

EXPERIMENT	DATE (1932)	GROUP I	GROUP II
I	Apr. 14-May 13	Basal	Basal
	May 13-June 6	Basal Plus 60,000 rat units Irradiated Ergosterol	Basal Plus 60,000 rat units Irradiated Yeast
	Aug. 12-Sept. 12	Basal	Basal
II	Sept. 12-Oct. 12	Basal Plus 120,000 rat units Irradiated Ergosterol	Basal Plus 120,000 rat units Irradiated Yeast

In Experiment I the irradiated ergosterol (corn oil solution) and the irradiated yeast were incorporated with the afternoon grain allowance, the amount of each required having been calculated from vitamin-D assays made in our own laboratory on these materials. In Experiment II the yeast and ergosterol were incorporated with the grain at each feeding (twice daily).³

During each of the last three days of each period indicated in Table I the entire milk production of each group was separated. The cream thus obtained was churned and the resulting butter rendered into pure fat. These fat samples were then stored in the dark in a cooler at 2° to 5° C. until assayed for vitamin D.

Blood samples were taken from the jugular vein of each cow one or two days before the close of a period, the samples from each group being combined immediately and dried over a steam bath.

On the last day of each period two defecations from each cow in each group were collected, combined, and dried in a hot air chamber.

The curative vitamin-D assays were made according to the standard "line test" procedure. The fat samples were melted and fed from a graduated pipette over a period of eight days. In some instances, particularly in the case of the samples from the control periods, the melted fat was incorporated in such amounts of the basal diet as were consumed in 7 to 8

³ We are indebted to Acetol Products, Inc., New Brunswick, N. J., for furnishing the irradiated ergosterol, and to Standard Brands, Inc., New York City, for furnishing the irradiated yeast.

days. The dried blood and dried feces samples were incorporated in the basal diet in like manner.

In the one prophylactic trial the fat was incorporated in the basal diet and a daily record kept of individual food consumption. The rats were chloroformed at the end of five weeks. The radii and ulnae were removed and preserved in formalin until observed for degree of calcification after staining the sections with silver nitrate. The femurs were removed, cleaned of adhering tissue, dried and crushed, extracted with alcohol and ether, and then ashed.

RESULTS

The data showing the critical levels of butterfat, *i.e.*, the amounts required to produce definite healing, are presented in table 2. It should be pointed out that many other levels of fat were fed and that several hundred rats were used in arriving at these critical values.

TABLE 2
Potency of Fat (Curative)

	GROUP	SUPPLEMENT FED TO COWS	NO. OF RATS	CRITICAL LEVEL OF BUTTERFAT	RAT UNITS PER GRAM OF FAT	% FAT IN MILK	R. U. PER QT.
Exp. I	I	None	13	6.0 gm.	0.17	3.4	5.7
	II	None	13	6.0 gm.	0.17	3.6	5.9
	I	60,000 Irrad. Erg.	11	480 mg.	2.08	3.6	72.8
	II	60,000 Irrad. Yeast	11	320 mg.	3.13	3.7	112.7
	Ia	None	4	7.5 gm.	0.13	4.2	5.3
	IIa	None	5	6.0 gm.	0.17	3.8	6.3
Exp. II	Ia	120,000 Irrad. Erg.	29	500 mg.	2.00	4.3	84.0
	IIa	120,000 Irrad. Yeast	24	300 mg.	3.33	4.1	133.2

It is very apparent, from table 2, that when 60,000 or 120,000 rat units of vitamin D either in the form of irradiated ergosterol or irradiated yeast were fed the vitamin-D content of the butterfat produced was markedly increased and that the increase in vitamin-D potency was greater when the irradiated yeast was fed than when the irradiated ergosterol was fed. The ratio between the vitamin-D potency of the ergosterol fats and the yeast fats was in the order of 2:3.2. That this was not a chance result was indi-

cated by the agreement between the results obtained in the two experiments in which different cows were used.

The results obtained curatively were substantiated and emphasized prophylactically. In table 3 it will be noted that a diet containing 0.25

TABLE 3
Potency of Fat (Prophylactic)

SUPPLEMENT FED TO COWS	LEVEL OF FAT FED TO RATS	NO. OF RATS	AV. DAILY FOOD INTAKE	AV. TOTAL FAT INTAKE	CALCI- FICA- TION*	BONE ASH
	<i>Pct.</i>		<i>Gm.</i>	<i>Mg.</i>		<i>Pct.</i>
120,000 Irrad. Erg.	0.25	6	7.7	674	0.25 +	29.98
120,000 Irrad. Yeast	0.25	6	7.8	683	1.17 +	37.06
120,000 Irrad. Erg.	0.50	6	6.9	1208	1.33 +	36.11
120,000 Irrad. Yeast	0.50	6	7.4	1295	2.58 +	40.24
120,000 Irrad. Erg.	0.75	6	7.3	1916	2.33 +	37.13
120,000 Irrad. Yeast	0.75	5	8.7	2284	3.20 +	46.42
Controls		6	6.6		0.0 +	25.90

* Width of Metaphysis: + = evidence of protection;

++ = fair protection;

+++ = good protection;

++++ = complete protection.

The numerical values were obtained by adding the plus signs of all rats in a group and dividing by the number of rats.

per cent of yeast fat resulted in as good calcification as was obtained with a diet containing 0.50 per cent of ergosterol fat and that 0.50 per cent of yeast fat was superior to 0.75 per cent of ergosterol fat. Some differences in total fat intake for the various groups will be noted. These were not large enough, however, to account for the difference in bone ash and calcification values. The greatest difference in total fat intake between two comparable groups (0.75 per cent level) was 368 milligrams. To obtain a difference of 1 per cent in the bone ash value of rats receiving, respectively, 0.50 per cent and 0.75 per cent of fat from the ergosterol-fed cows, it required 708 milligrams of fat; in the case of comparable groups receiving fat from yeast-fed cows it required 989 milligrams of fat to increase the bone ash value 6.18 per cent.

The close relationship known to exist between blood and milk prompted investigation of the vitamin-D content of the blood. It might be expected,

in view of the difference in vitamin-D potency of the two milks, that a difference would be found in the vitamin-D potency of the blood of the cows producing these milks. Such was not the case, according to the data shown in table 4. This would suggest a difference in absorption of vitamin

TABLE 4
Potency of blood (dry)

GROUP	SUPPLEMENT FED TO COWS	NO. OF RATS	CRITICAL LEVEL OF BLOOD	RAT UNITS PER GRAM OF BLOOD
I	None	15	500 mg.	2.0
II	None	15	500 mg.	2.0
I	60,000 Irrad. Erg.	5	175 mg.	5.7
II	60,000 Irrad. Yeast	6	200 mg.	5.0
Ia and IIa	None	8	300 mg.	3.3
Ia	120,000 Irrad. Erg.	9	150 mg.	6.7
IIa	120,000 Irrad. Yeast	9	150 mg.	6.7

D from the intestinal tract, yet no indication of this is apparent from the vitamin-D content of the feces (Table 5).

TABLE 5
Potency of feces (dry)

GROUP	SUPPLEMENT FED TO COWS	NO. OF RATS	CRITICAL LEVEL OF FECES	RAT UNITS PER GRAM OF FECES (DRY)
I	60,000 Irrad. Erg.	8	350 mg.	2.9
II	60,000 Irrad. Yeast	8	350 mg.	2.9
Ia and IIa	None	25	10.0 gm.	0.10
Ia	120,000 Irrad. Erg.	8	250 mg.	4.00
IIa	120,000 Irrad. Yeast	8	250 mg.	4.00

DISCUSSION

Some question may be raised regarding the failure of doubling the intake of vitamin D to bring about a greater increase in the vitamin-D content of the fat (Table 2). This failure can be partially explained on the basis of total fat production since, according to Hess, Light, Frey, and Gross (11), the lower the total production of butterfat the higher will be

the concentration of vitamin D in the fat. The average daily milk production of both groups of cows in both experiments was much the same but in Experiment I the fat production was less than in Experiment II (Table 6).

TABLE 6
Milk and fat production

PERIOD	GROUP	COW NO.	AV. DAILY MILK	% FAT	DAILY FAT
Experiment I					
I Apr. 14- May 13	I Basal	2	Lb. 30.4	3.64	Lb. 1.11
		380	34.0	3.00	1.02
		381	30.5	3.70	1.13
				3.44*	
	II Basal	1	33.0	3.41	1.13
		3	33.8	3.55	1.20
		382	32.4	3.86	1.25
				3.61	
	I Basal + 60,000 units Irradiated Ergosterol	2	31.0	3.65	1.13
		380	32.1	3.32	1.07
381		25.3	3.92	0.99	
II May 13- June 6				3.61	
	II Basal + 60,000 units Irradiated Yeast	1	34.1	3.46	1.18
		3	26.0	3.85	1.00
		382	31.8	3.86	1.23
				3.71	
	Experiment II				
I Aug. 13- Sept. 12	Ia Basal	351	33.3	4.62	1.54
		373	32.3	4.44	1.43
		386	28.1	3.36	0.94
				4.17	
	IIa Basal	352	42.9	3.80	1.63
		371	32.6	4.08	1.33
		384	25.5	3.28	0.84
				3.76	
	Ia Basal + 120,000 units Irradiated Ergosterol	351	31.5	4.57	1.44
		373	32.3	4.70	1.52
386		25.3	3.50	0.89	
II Sept. 12- Oct. 12				4.32	
	IIa Basal + 120,000 units Irradiated Yeast	352	37.2	4.10	1.53
		371	33.5	4.40	1.47
		384	22.6	3.80	0.86
				4.14	

* Total fat production of the group divided by total milk production of the group multiplied by 100.

In Experiment II it was intended originally to make the assays on cream. In order to preserve the cream 0.5 cc. of formaldehyde was added per quart. The assays on the cream did not prove satisfactory and after a period of two months the preserved cream was churned and rendered into fat. The critical levels given in table 2 are based on the assays made on fat so prepared. It was at first thought that the preservation of the cream with formalin may have destroyed some of the vitamin D. However, several samples of fat prepared from cream to which from 1.0 to 2.0 cc. of formalin per quart had been added, showed no lowered vitamin-D potency when compared with samples prepared identically from unpreserved cream. One of these samples was prepared from a cow receiving 120,000 rat units of vitamin D in the form of irradiated yeast and showed a potency of at least 200 units per quart. We are unable to explain the low potencies obtained in Experiment II but feel that the relationship shown between the two fats is valid. Thus the contention of Hess, *et al.* (9) that cows transfer the vitamin D of irradiated yeast to milk more efficiently than they do that from irradiated ergosterol is substantiated although the difference between the efficiencies is, according to our data, more nearly 3:2 than 3:1 as reported by Hess, *et al.*

According to Frey, Light, and Wilson (12) the rate of secretion of vitamin D into the milk is roughly proportional to the concentration of vitamin D in the blood. The rather small difference in concentration of vitamin D in the blood in Experiments I and II is in line with the potencies of the butterfats, as reported in table 2, in spite of the fact that in Experiment I the vitamin supplements were fed in the afternoon whereas in Experiment II they were fed both in the forenoon and afternoon. In both experiments the blood samples were drawn between 8 and 10 A.M.

In a previous paper (10) it was shown that only a small percentage of the vitamin D intake of cows is transferred to the milk. Other workers (11) (13) (14) have reported similar findings. From the data in table 5 it is apparent that a tremendous amount of vitamin D is excreted through the intestinal tract, but apparently there was no difference in the concentration of vitamin D in the feces of the ergosterol- and yeast-fed cows. This would eliminate any correlation between differences in absorption of vitamin D and efficiency of transfer to the milk.

It is quite possible that in the cows receiving irradiated ergosterol there was a greater storage of vitamin D in the liver and other tissues of the body. This would be in accordance with the recent work of Russell, Taylor, and Wilcox (15) whose results demonstrated that the failure of irradiated ergosterol to equal cod-liver oil in promoting bone formation in the chicken is not associated with any failure of the factor in ergosterol to enter certain tissues of the body. Also, McCoord and Luce-Clausen (16), in their study of vitamin A storage in the livers of rats, came to the conclusion

that the concentration of vitamin A in the blood is no indication of the amount that may be stored in the liver.

The possibility remains, as pointed out in the recent work of Bethke, Record, and Kennard (8) that species difference plays a large part in determining the efficiency with which a particular vitamin-D source is utilized. In their work it was shown conclusively that irradiated ergosterol and irradiated yeast were considerably less effective than the rat equivalent amount of cod liver oil for calcification in chicks. To attribute our results to the presence of different forms of vitamin D in irradiated ergosterol and irradiated yeast, both of which were equally effective in rats but not in cows, would be as safe a speculation as could be made with the data at hand. The data do suggest that the loss in efficiency of the irradiated ergosterol occurs in the transformation from blood to milk.

It is worthy of mention, in this connection, that some of our unpublished data indicate that the vitamin D in cod liver oil is somewhat more efficiently transferred to eggs than is that in irradiated ergosterol.

SUMMARY

Two groups of lactating Holstein cows were fed 60,000 rat units of vitamin D as irradiated ergosterol and 60,000 rat units of vitamin D as irradiated yeast daily. Two other groups of lactating Holstein cows were fed 120,000 rat units of vitamin D from the same sources daily.

Vitamin D assays of the milk produced by these groups of cows showed that the irradiated ergosterol was approximately two-thirds as efficient in allowing transfer of its vitamin D to the milk as was the irradiated yeast. No satisfactory explanation for this was found. It could not be attributed to a difference in absorption from the intestinal tract since the vitamin-D content of the feces and of the bloods was the same regardless of the supplement fed. The existence of different forms of vitamin D having different species effects is considered as a possible explanation, as is also a difference in the "disappearance" into the tissues of vitamin D from the two sources.

REFERENCES

- (1) HESS, A. F., WEINSTOCK, M., AND RIVKIN, H. Some differences in action between irradiated ergosterol and cod liver oil. *Proc. Soc. Exp. Biol. and Med.* 27: 665-666. 1930.
- (2) HESS, A. F., AND LEWIS, J. M. An appraisal of antirachitics in terms of rat and clinical units. *Jour. Amer. Med. Assoc.* 101: 181-184. 1933.
- (3) MUSSEHL, F. E., AND ACKERSON, C. W. Irradiated ergosterol as an antirachitic for chicks. *Poultry Sci.* 9: 334-338. 1930.
- (4) MASSENGALE, O. N., AND NUSSMEIER, MILDRED. The action of activated ergosterol in the chicken. II. The prevention of leg weakness. *Jour. Biol. Chem.* 87: 423-426. 1930.
- (5) HESS, A. F., AND SUPPLEE, G. C. The action of irradiated ergosterol on rats and chickens. *Proc. Soc. Exp. Biol. and Med.* 27: 609-610. 1930.

- (6) RUSSELL, W. C., AND KLEIN, D. The vitamin-A content of a ration and the anti-rachitic potency of irradiated ergosterol. *Poultry Sci.* 10: 269-274. 1931.
- (7) STEENBOOK, H., KLETZIEN, S. W. F., AND HALPIN, J. G. The reaction of the chicken to irradiated ergosterol and irradiated yeast as contrasted with the natural vitamin D of fish liver oils. *Jour. Biol. Chem.* 97: 249-264. 1932.
- (8) BETHKE, R. M., RECORD, P. R., AND KENNARD, D. C. The antirachitic efficiency of irradiated ergosterol, irradiated yeast, and cod liver oil for the chicken. *Jour. Nutrition* 6: 413-425. 1933.
- (9) HESS, A. F., LEWIS, J. M., MACLEOD, F. L., AND THOMAS, B. H. Antirachitic potency of the milk of cows fed irradiated yeast or ergosterol. *Jour. Amer. Med. Assoc.* 97: 370-375. 1931.
- (10) KRAUSS, W. E., BETHKE, R. M., AND MONROE, C. F. The effect of feeding irradiated ergosterol to cows on the vitamin-D content of milk. *Jour. Nutrition* 5: 467-477. 1932.
- (11) HESS, A. F., LIGHT, R. F., FREY, C. N., AND GROSS, J. A study of the milk, blood, and excreta of cows fed moderate and excessive amounts of irradiated yeast or ergosterol. *Jour. Biol. Chem.* 97: 369-377. 1932.
- (12) FREY, C. N., LIGHT, R. F., AND WILSON, L. T. Secretion of certain constituents of milk—vitamin D. Part II. *Certified Milk* 9: 10-11, No. 94. 1934.
- (13) HART, E. B., STEENBOOK, H., TEUT, E. C., AND HUMPHREY, G. C. Dietary factors influencing calcium assimilation. XI. The influence of cod liver oil upon calcium metabolism of milking cows. *Jour. Biol. Chem.* 84: 359-376. 1929.
- (14) HART, E. B., STEENBOOK, H., KLINE, O. L., AND HUMPHREY, G. C. Dietary factors influencing calcium assimilation. XIII. The influence of irradiated yeast on the calcium and phosphorus metabolism of milking cows. *Jour. Biol. Chem.* 86: 145-155. 1930.
- (15) RUSSELL, W. C., TAYLOR, M. W., AND WILCOX, D. E. The fate of the antirachitic factor in the chicken. III. The distribution of the factor from cod liver oil and from irradiated ergosterol in certain tissues of the chicken. *Jour. Biol. Chem.* 105: lxxiv-lxxv. 1934.
- (16) MCCOORD, AUGUSTA B., AND LUCE-CLAUSEN, ETHEL M. The storage of vitamin A in the liver of the rat. *Jour. Nutrition* 7: 557-572. 1934.

ADDENDA

Since the above manuscript was submitted there appeared an article with an almost identical title (Russell, W. C., Wilcox, D. E., Waddell, J., and Wilson, L. T. The relative value of irradiated yeast and irradiated ergosterol in the production of vitamin D milk. *JOUR. DAIRY SCI.* 17: 445-453. 1934). These two articles are essentially in agreement except that in our work the difference in efficiency was demonstrated at the lower as well as the higher level of vitamin D intake.

THE EFFECT OF FEEDING RAW ROCK PHOSPHATE ON THE FLUORINE CONTENT OF THE ORGANS AND TISSUES OF DAIRY COWS

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The influence of fluorine ingestion upon the distribution and storage of fluorine in the animal body has been little studied. This is no doubt due to the fact that adequate quantitative methods for the estimation of fluorine have been wanting, or that available methods were extremely tedious and difficult to use. With the development of methods for the estimation of fluorine the problem becomes a practical one in the study of fluorine metabolism. The early investigations dealt with the distribution of fluorine in laboratory animals. Little work has been reported on the distribution and storage of fluorine in farm animals. This is of practical importance to man because of its relation to the quantities of fluorine present in meat products, particularly where feeds have been used which contain abnormally high quantities of fluorine.

A series of fluorine determinations were made on the tissues and organs of dairy cows fed various levels of raw rock phosphate containing 3.5 per cent fluorine. We wished to follow the distribution of fluorine in the tissues of the normal dairy cow; to determine in what tissues fluorine storage took place; and also to determine the influence of fluorine ingestion upon the normal distribution of fluorine in the tissues.

The presence of fluorine in the tissues of the animal body has been known for a long time. Knowledge concerning the fluorine content of normal animal tissues and the influence of fluorine ingestion upon the storage of fluorine in the body is quite meager. Gautier and Clausmann (7) have pointed out that fluorine was found by them in all tissues. These investigators, Bethke, Kick, Edgington and Wilder (2), Boissevain and Drea (3), as well as others have reported the fluorine content of teeth and bones. Bethke and coworkers reported approximately 0.0409 and 0.0231 per cent fluorine in the femurs of swine. Boissevain and Drea determined fluorine spectrographically in human teeth and reported 0.025 and 0.06 per cent

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fluorine in the enamel and dentine, respectively. Contamination of the drinking water with 2 parts of fluorine per million resulted in a significant increase in the quantity of fluorine in the enamel and dentine of the teeth. An increase in the fluorine content of the bones was found when fluorine was present in the drinking water. These investigators were unable to detect fluorine in other tissues or in milk. Brandl and Tappeiner (4), Sonntag (10), and Bethke *et al.* have also demonstrated that the feeding of fluorine increases its storage in the animal body, particularly in the bones. The fluorine content of the bones of adults has been shown to be greater than those of the young by Gautier and Clausmann (8). Wrampelmeyer (12) reported an increase in the fluorine content of adult teeth over that of the child. Gautier and Clausmann's results for dental enamel range from 0.118 to 0.180 per cent. These results are similar to those of Carnot (5) who reports 0.10 to 0.30 per cent fluorine in dental enamel. For other references the reader is referred to the reviews of DeEds (6) and McClure (9).

EXPERIMENTAL

The animals used for this study were Holstein dairy cows which had been placed on experiment as young heifers 4-6 months of age and continued thereon until after the third calving. Eighteen heifers were divided into 6 lots of three animals each. Lot I received a grain mixture, corn silage and alfalfa hay. Lot II received a grain mixture, corn silage and timothy hay. Lot III received timothy hay, corn silage and a grain mixture to which was added 2.50 per cent special steamed bone meal. Lots IV, V and VI received the basal ration of lot III with various levels of raw rock phosphate replacing a like quantity of the bone meal. Thus lot IV received 0.625 per cent rock phosphate, or 0.022 per cent fluorine, lot V received 1.25 per cent rock phosphate, or 0.044 per cent fluorine, and lot VI received 2.50 per cent rock phosphate, or 0.088 per cent fluorine. The same grain mixture was used throughout the experiment and the feeds were varied in amount to care for the needs of the growing animal and for lactation according to the Morrison feeding standards. Ground limestone was added to the rations of lots III to VI inclusive in order to maintain a comparable Ca:P ratio in all lots. The animals were removed individually from the experiment at the approximate age of five years. Only one animal from each of lots I to III was taken for analytical investigation, hence the control group represents 3 animals only.

Samples of teeth, bone, liver, kidney, heart muscle, pancreas, thyroid, hair and tendon were obtained. The teeth were divided into two sets. The incisor teeth were used for analyses of enamel and dentine. The outer enamel was carefully removed with a hand file, the tooth was then burrished on an emery stone to remove all traces of enamel and the remainder

of the tooth was used as representative dentine. All of the incisor teeth from each cow were thus used in making up a sample. The molar teeth from the right jaw were removed, the roots broken off and the crown of the tooth analyzed for its fluorine content. Due to the structure of cattle molars the amount of enamel present is not restricted to the outer layer of enamel but invaginations of enamel occur between the cusps of the tooth. Hence analyses of the molars should give values lying between enamel and dentine. The fat of the bones was extracted by hot alcohol. All tissues were dried in a drying room for 6 to 7 days. They were then removed, weighed, ground and stored in screw top sample bottles.

In the case of the hoof and hair scrubbing with a brush, soap and distilled water was resorted to in order to free them of contamination from excreta. The sample of hair was obtained from the tail. Skin and fat were obtained only in the case of normal animals. The skin was carefully shaved and washed free of adherent material while the fat free of connective tissue was saponified in order to make sure that the fluorine present, if any, could be held with Na_2CO_3 during the ashing process.

The method used in these analyses was that of Willard and Winter (11) as modified by Armstrong (1). Precautions were used to obtain proper ashing as we found that even small traces of carbon interfered with recovery of added fluorine. To obtain a white ash we adopted the following procedure. The sample was moistened thoroughly with enough Na_2CO_3 solution to incorporate from 1 to 4 gms. of Na_2CO_3 , depending upon the size of the sample. The sample was then completely dried on a hot plate and ashed at a low temperature (about $650^\circ \text{C}.$) for not more than 20 minutes and usually only 10. The sample was then moistened with redistilled water, again dried and ashed. This was repeated until a white ash was obtained. From three to five washings were found to be necessary for most of the tissues.

The entire distillate was collected and titrated, using freshly prepared alizarin as an indicator. A determination of a blank containing all of the reagents was made at frequent intervals and the blank deducted from the titration of the samples. In our experience excellent results were obtained with this method when due precautions were observed with respect to ashing and titrating, and when small quantities requiring less than 10 cc. of $\text{Th}(\text{NO}_3)_4$ were used.

Analyses were made in duplicate for all but one cow in each lot. One cow was selected from each lot and quadruplicate samples were taken for cows No. 62, 66, 69, and 71. Two duplicate determinations were made without added fluorine while 0.532 mg. of fluorine was added to the remaining two. Thus the analyses of the tissues from one cow from each lot were completely checked by fluorine recoveries.

TABLE 1
The fluorine content of dairy cattle tissues expressed in mgs. per 100 gm. dry weight

	COW NO.	TEETH			BONE	LIVER	KIDNEY	HEART MUSCLE	PANCREAS	THYROID	TENDON	HAIR	HOOF
		Enamel	Dentine	Molar									
Controls—no added fluorine	57	22.50	44.90	52.90	60.05	0.55	0.69	0.23	0.69		0.54	0.61	
	60	25.91	42.40	55.02	58.22	0.58	0.89	0.27	1.03			0.61	0.72
	62	31.57	99.45	53.35	56.92	0.52	1.01	0.27	0.85	0.68	0.75	0.70	
	Ave.	26.66	62.25	53.76	58.40	0.55	0.86	0.26	0.86	0.68	0.65	0.64	
Lot IV—added fluorine (0.022 per cent of the grain mixture)	64	291.08	399.60	381.02	544.92	0.78	3.18	0.48	0.70	68.66		1.43	
	65	264.48	410.20	382.28	533.57	0.85	2.57	0.37	0.82	108.60		1.17	
	66	290.42	477.00	392.92	527.82	0.53	2.55	0.88	0.95			1.34	
	Ave.	281.99	428.93	385.41	535.43	0.72	2.77	0.58	0.84	88.63		1.31	
Lot V—added fluorine (0.044 per cent of the grain mixture)	67	301.72	531.20	476.53	635.36	0.73	3.47	0.75	0.83			0.93	0.82
	68	332.12	454.50	465.88	623.96	0.83	3.35	0.64	0.90			1.06	
	69	296.00	561.87	483.36	661.72	0.62	3.82	0.97	0.92				
	Ave.	309.95	515.86	475.26	640.35	0.73	3.55	0.75	0.88			1.00	
Lot VI—added fluorine (0.088 per cent of the grain mixture)	70	685.52	777.50	708.32	945.44	0.85	4.30	0.94	0.91	164.20	1.12	1.39	
	71	690.70	865.38	709.84	938.35	0.77	4.37	0.87	1.06		1.15	1.41	1.16
	72	688.56	839.50	726.56	965.20								
	Ave.	688.29	827.46	714.87	949.60	0.81	4.34	0.91	0.98	164.20	1.14	1.40	

RESULTS

It is seen in table 1 that all of the tissues examined contained traces of fluorine. Bone contained on the average more fluorine than other tissues, although dentine was only slightly less. These structures including enamel apparently form the greatest place of storage of fluorine. The active organs such as the liver, heart muscle, pancreas, thyroid, kidney, tendon and hair were relatively free of fluorine as compared to bone for example. With the exception of the pancreas, and possibly the liver, each added increment of fluorine to the ration resulted in an increased storage of fluorine. This supports the work of Brandl and Tappeiner (4), Sonntag (10), Bethke *et al.* (2), and Boissevain and Drea (3).

In cattle the fluorine content of the dentine is higher than it is in the enamel. In this respect these analyses corroborate those of Boissevain and Drea. Their results show that the dentine contains nearly twice the amount of fluorine determined spectrographically as the enamel of human teeth. Our results obtained for cattle show 0.0266 per cent and 0.0625 per cent, respectively, for normal enamel and dentine. These values approximate those reported for human teeth by Boissevain and Drea. They are below the values reported for enamel by Gautier and Clausmann and by Carnot. The values obtained for normal bone lie within the range of fluorine content reported by Gautier and Clausmann. They are somewhat higher than the results obtained by Bethke *et al.* for swine.

It was impossible for us to demonstrate large amounts of fluorine in washed hair.

The results obtained in these studies show that additional fluorine in the normal ration increases the quantity of fluorine in the tissues. The most noteworthy increase in fluorine content was found in the bones, teeth, and thyroid. The latter increased its fluorine content 24 times on the high level of fluorine feeding. The amount of fluorine in the enamel increased 25 times over that of the normal when 0.088 per cent of fluorine was added to the grain ration. In the bones the increased quantity of fluorine was only 16 times that of the normal when 0.088 per cent fluorine was included in the grain mixture. The general relationship between the structures with respect to their fluorine content remained the same, namely, in the order of bone, dentine, and enamel. The remaining tissues with the exception of the pancreas and liver showed an increased fluorine content of a much

The results obtained by these studies suggest that fluorine storage closely follows the deposition of calcium and phosphorus. They also indicate that fluorine is not effectively excreted from the body. It is stored, perhaps as a means of removing it from circulation. It seems unlikely that sufficient fluorine can be stored or held in the meat to become a potential source of danger to the public health even when definitely chronic toxicosis has developed.

SUMMARY AND CONCLUSIONS

Fluorine was found to occur in all of the normal tissues studied. The greatest quantity of fluorine was found to accompany calcium and phosphorus deposition. Thus the bones and teeth contained large quantities of fluorine while the more active organs such as the liver, kidney, heart muscle, and the other tissues studied showed only small quantities of fluorine.

The quantity of fluorine present in normal dentine and normal bone of dairy cows was found to be similar and the average fluorine content was found to lie between 42 and 63 milligrams per 100 grams of dried tissue. The fluorine content of the liver, kidney, heart muscle, pancreas, thyroid, tendon, hair, and hoof was found to be less than 1 milligram per 100 grams of dried normal tissue. The inclusion of 0.088 per cent fluorine in the grain mixture resulted in an increased storage of fluorine in the bones and teeth. This amounted to 16 to 25 times that found in normal osseous structures. The internal organs, tendon, and hair likewise doubled in fluorine content when the grain mixture contained 0.088 per cent fluorine.

REFERENCES

- (1) ARMSTRONG, W. D. Modification of the Willard-Winter method for fluorine determination. *J. Am. Chem. Soc.* 55: 1741. 1933.
- (2) BETHKE, R. M., EDGINGTON, B. H., AND WILDER, O. H. The effect of feeding sodium fluoride and rock phosphate on bone development in swine. *Proc. Am. Soc. Animal Prod.* 29: 29. 1929.
- (3) BOISSEvain, C. H., AND DREA, W. F. Spectroscopic determination of fluorine in bones, teeth and other organs in relation to fluorine in drinking water. *Jour. Dent. Res.* 13: 495. 1933.
- (4) BRANDL, J., AND TAPPEINER, H. Ueber die ablagerung von fluorverbindungen im organismus nach Fütterung mit Fluornatrium. *Ztschr. f. Biol.* 28: 518. 1891.
- (5) CARNOT, A. Recherche du fluor dans les os moderne et les os fossiles. *Compt. Rend.* 114: 1189. 1892.
- (6) DEEDS, F. Chronic fluorine ingestion. *Medicine* 12: 1. 1933.
- (7) GAUTIER, A., AND CLAUSMANN, P. Le fluor dans l'organisme animal. *Compt. Rend. Acad. Sci.* 156: 1425. 1913.
- (8) GAUTIER, A., AND CLAUSMANN, P. Le fluor dans l'organisme animal. *Compt. Rend. Acad. Sci.* 156: 1347. 1913.
- (9) MCCLURE, F. J. A review of fluorine and its physiological effects. *Physiol. Reviews* 13: 277. 1933.
- (10) SONNTAG, G. Über ein Verfahren zur Bestimmung des Fluorgehalt von Knochen und Zähnen normaler and mit Fluoriden gefütterter Hunde. *Chem. Zentr.* 87: 1095. 1916.
- (11) WILLARD, H. H., AND WINTER, O. B. Volumetric method for the determination of fluorine. *Ind. and Eng. Chem. Analyt. Ed.* 5: 7. 1933.
- (12) WRAMPMEYER, E. Ueber den Fluorgehalt der Zähne. *Zeitschr. f. Analytical Chem.* 37: 550. 1893.

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SOME PHYSICO-CHEMICAL PROPERTIES OF LACTOSE

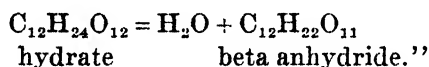
V. THE INFLUENCE OF OTHER SUBSTANCES UPON THE EQUILIBRIUM ROTATION OF LACTOSE

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INTRODUCTION

In 1903, Hudson (7) suggested that the mutarotation of lactose was due to a dynamic equilibrium between the two forms. He assumed that these two forms were beta anhydride and lactose hydrate. In 1904, he published another paper (8) in which he stated that, "... the mutarotation of milk sugar results from the slow establishment in cold solution of the balanced reaction,



In 1910, he published another paper (9) asserting that the mutarotation was due to a slow reversible transformation of the hydrated sugar into a lactone. In his papers on lactose, he assumed that lactose hydrate was the aldehydrol form, and that when alpha anhydride was dissolved in water, it was changed at once into this form. However, he admitted that there was no direct evidence to show that the freshly dissolved hydrate existed in the hydrated condition.

Gillis (4) has denied that hydrated lactose is the hydrated aldehyde related equally to both alpha and beta lactose, and in the opinion of the writer, Gillis is correct. The fact that hydrated lactose is a hydrate of alpha does not necessarily eliminate the reaction given above as a possible explanation of the mutarotation. However, if this equation does represent conditions as they exist in solution, then the equilibrium should vary with the water concentration and this fact should be susceptible of experimental demonstration.

Some information is available in the literature which has a bearing on this problem. Browne (2, p. 188) in writing of the equilibrium rotation of sugars stated, "Highly concentrated solutions, however, do not always

give the true end rotation; such solutions must first be diluted and then allowed to stand for the change in rotation to be completed." This does not seem to be true of lactose. The United States Bureau of Standards has reported (17) that the specific rotation of lactose remains unchanged at all concentrations. It may be argued, of course, that because of its low solubility, solutions of lactose are not sufficiently concentrated for changes in the water concentration to be evident.

In 1880, Tollens (13) found that the specific rotation of sucrose was raised slightly in solutions of ethyl or methyl alcohol as well as in solutions of acetone. However, sucrose is not a mutarotating sugar. In 1895, Tanret (12) reported that the specific rotation of glucose varied with the concentration, increasing from 52.5 to 55.0 in the most concentrated solution examined. The specific rotation was also increased in solutions of alcohol. Trey (14) also reported that the specific rotation of glucose was increased in solutions of methyl alcohol. Trey (16) found that the specific rotation of lactose was decreased in solutions of ethyl or methyl alcohol and in solutions of acetone. Holty (5) found that the specific rotation of lactose was decreased in pyridine solutions, but the decrease was less when the concentration of lactose was high. However, Pucher and Dehn (11) claimed that lactose combines with pyridine, and therefore interpretation of Holty's data is difficult.

It is quite evident that the experimental results seem to support Hudson's theory as expressed by his equation. However, before considering the question as settled, an attempt was made to gather additional evidence. It was desired to lower the water concentration as much as possible and determine the effect upon the alpha-beta equilibrium. Lactose is practically insoluble in anhydrous alcohol but it is appreciably soluble in glycerol. Glycerol was therefore chosen as the solvent to be used in this investigation. Experiments were also planned to determine whether such salts as calcium chloride, which would bind part of the water in the solution, might alter the equilibrium between the mutarotating forms.

The mere fact that the specific rotations of lactose in glycerol and in water were different would not constitute proof that a shift in the equilibrium had taken place. However, if the equilibrium in glycerol were not the same as in water, then it should be possible to detect mutarotation after diluting the glycerol solutions with a large volume of water. This test was employed in the following experiments.

EXPERIMENTS

Three solutions, each containing 1.500 grams of lactose, were prepared. The solvents used were: 1, 25 grams of c.p. glycerol; 2, 25 grams of c.p. glycerol + 70 cc. of water; 3, 70 cc. of water. The lactose was dissolved by heating the flasks in hot water. Then, the solutions were cooled to room

temperature, diluted to 110 cc. with water, and read as quickly as possible in a 400 mm. water jacketed polariscope tube at 26° C. The solution equilibrated in glycerol gave a rotation of 3.03°; the solution equilibrated in aqueous glycerol gave a reading of 2.80°; the solution equilibrated in water gave a reading of 2.83°. When the first solution was warmed at 80° C. for five minutes and then reexamined in the polariscope, the rotation was found to be 2.80°.

The high value found for the glycerol solution was open to suspicion since the solution was prepared from the form of lactose having high rotation. Therefore, the experiment was repeated starting with beta anhydride, and taking particular care that equilibrium should be established before diluting with water. The glycerol was freed from water by heating to 150° C., under reduced pressure. Beta lactose was then dissolved in it by heating to 120° C. The solution was held at 100° C., for 48 hours. After cooling, the solution was diluted with four volumes of water, and examined at once in a polariscope. The first reading was 6.57, the final rotation was 6.15. It is of interest to note that the fall in rotation is 6.4 per cent in this experiment and 6.6 per cent in the previous one. Therefore, we may safely assume that equilibrium had been established in both experiments.

These experiments indicated that in glycerol there was a shift toward the more strongly rotating form of lactose, although the reverse is reported for alcoholic solutions. In aqueous solutions, such a shift normally occurs at elevated temperatures. If this is true in glycerol, and if equilibration is very slow in glycerol, there is a possibility that, in spite of slow cooling, the measurements really apply to the equilibrium at some high temperature which was "frozen" on cooling. This might have caused an error in these experiments, but that is not believed to be the case.

Somewhat similar experiments were carried out using solutions of salts and in most cases the equilibrium rotation was raised. An increase in rotation cannot be explained in terms of the original equation. Therefore, it seemed that some complicating factor was present. If this were true, then the data, obtained in the study of salt solutions, could not be used to solve the original problem regarding the condition of lactose in solution. Therefore, that part of the investigation was discontinued for the time being.

Trey (16) studied the mutarotation of lactose in salt solutions. He was interested primarily in the velocity of mutarotation, but incidentally he showed that some salts influence the equilibrium rotation. In order to determine if this was a general property of all salts, weighed amounts of various salts were placed in 50 cc. volumetric flasks. Twenty-five cc. of a concentrated solution of lactose was then added to each flask, with sufficient water to bring the solution almost to the mark. After the salt had dissolved completely, the volume was adjusted to 50 cc. and then the flasks

were heated in hot water to approximately 70° C. They were allowed to cool slowly to room temperature and finally examined in a polariscope using a water jacketed tube. The data are shown in Table 1. After making the initial observation, some of the solutions were diluted with water and placed in the polariscope. The diluted solutions exhibited mutarotation.

TABLE 1
The influence of various salts on the equilibrium rotation of lactose

SALT USED	GRAMS OF SALT	CC. OF SUGAR SOLUTION	ROTATION	SHIFT
First series				
None	0	25	20.34	
BaCl ₂	10	25	21.40	+ 1.06
Ca(NO ₃) ₂ · 4H ₂ O	20	25	22.13	+ 1.79
Second series				
None	0	25	20.14	
KI	10	25	20.25	+ 0.11
NaNO ₃	10	25	20.93	+ 0.79
Ca(NO ₃) ₂ · 4H ₂ O	15	25	21.20	+ 1.06
Third series				
None	0	25	10.07	
MgCl ₂ · 6H ₂ O	15	25	10.28	+ 0.21
BaCl ₂ · 2H ₂ O	15	25	10.63	+ 0.56
HgCl ₂	3	25	10.09	+ 0.02
KCl	15	25	9.78	- 0.29
Fourth series				
None	0	25	28.24	
NaCl	12	25	29.37	+ 1.13
K ₂ SO ₄ · 2H ₂ O	15	25	25.97	- 2.27
NaBr	15	25	29.16	+ 0.92
NH ₄ Cl	14	25	27.30	- 0.94

These experiments showed that practically all salts, if sufficiently concentrated, influence the equilibrium rotation of lactose, but the effect is not uniform. Of the salts examined, all except potassium sulphite, and the chlorides of ammonium and potassium, raised the final rotation. These three salts lowered it. Trey (15) definitely stated that he did not believe that the abnormal mutarotations which he observed in salt solutions were due to compounds of sugar and salt existing in solutions. He points out that even though, in some cases, such compounds are known in the solid state, there is no proof that they exist in solution. The view that lactose combines to some extent with practically all salts in solution is accepted with reluctance. However, no other theory seemed to be quite so simple, and it was thought desirable either to prove or disprove it.

If the abnormal equilibrium rotation is due to the formation of a compound of lactose and a salt, then by increasing either the lactose concentration, or the salt concentration, compound formation should be favored. As the salt concentration increases, the rotation should rise steadily and approach a constant value, which would be reached when all of the sugar is in the form of the compound. As the lactose concentration increases, the total deviation should increase, but the actual specific rotations would not reach the high value that was found in the first case. Furthermore, the specific rotation might even pass through a maximum and then decrease again. This is necessarily true, since there must always be present a large excess of free sugar. These predictions were found to fit the three cases which were examined in detail.

Concentrated solutions of calcium chloride, calcium nitrate, and potassium chloride were prepared from Baker's Analyzed chemicals. Their concentrations were not determined exactly since they did not seem of particular importance. A series of solutions were prepared from the stock salt solutions, pure lactose, and distilled water. In each case, the final volume was adjusted to 50 cc. The solutions were heated for a minute or two in boiling water to establish equilibrium. Then they were cooled slowly, and examined in a polariscope. The data are shown in Tables 2 and 3.

TABLE 2

The effect of salt concentration upon the equilibrium rotation of lactose

CC. OF CaCl_2 SOLUTION	GRAMS OF LACTOSE	ROTATION	ROTATION PER GRAM	TOTAL DEVIATION
0	2.000	8.23	4.11	
10	2.000	8.67	4.33	+ 0.44
20	2.000	8.86	4.43	+ 0.63
30	2.000	9.19	4.59	+ 0.96
40	2.000	9.40	4.70	+ 1.17
to 50 cc.	2.000	9.66	4.83	+ 1.43
0	4.000	16.36	4.09	
10	4.000	17.15	4.29	+ 0.79
20	4.000	18.20	4.54	+ 1.84
30	4.000	18.92	4.73	+ 2.56
40	4.000	19.17	4.79	+ 2.81
50	0	0		

The calcium chloride solution contained 43.95% of anhydrous salt by weight.

A study of these tables shows that the shift in specific rotation depends upon the concentration of both salt and sugar in the way which would be expected if a compound did exist in solution. The degree of association cannot be estimated without knowing the specific rotation of the compound itself.

TABLE 3

The variation of the equilibrium rotation in salt solution with change in lactose concentration

SALT USED	CC. OF SALT SOLUTION	GRAMS OF LACTOSE	ROTATION	ROTATION PER GRAM	TOTAL DEVIATION
Calcium nitrate	0	7.000	28.32	4.05	
“ “	10	2.000	8.43	4.22	+ 0.33
“ “	20	2.000	8.69	4.35	+ 0.59
“ “	20	4.000	17.57	4.39	+ 1.37
“ “	20	8.000	35.22	4.40	+ 2.82
“ “	40	2.000	9.43	4.72	+ 1.33
“ “	40	4.000	19.05	4.76	+ 2.85
“ “	40	8.000	38.38	4.79	+ 5.98
Calcium chloride	10	4.000	16.90	4.23	+ 0.70
“ “	20	2.000	8.53	4.27	+ 0.43
“ “	20	4.000	17.71	4.43	+ 1.51
“ “	20	8.000	35.77	4.47	+ 3.37
“ “	40	2.000	9.13	4.57	+ 1.03
“ “	40	4.000	18.43	4.61	+ 2.23
“ “	40	8.000	38.33	4.78	+ 5.93
Potassium chloride	to 50 cc.	1.000	3.94	3.94	- 0.11
“ “	“	3.000	11.90	3.97	- 0.25
“ “	“	5.000	19.88	3.98	- 0.37
“ “	“	7.000	27.84	3.98	- 0.41

Calcium nitrate solution: 400 grams $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ plus 250 cc. of water.

Calcium chloride solution: 570 grams of anhydrous CaCl_2 per liter.

Potassium chloride solution: 340 grams plus 1000 cc. of water.

The results of these experiments support the theory that lactose forms compounds with salts in solutions. The literature of lactose gives little information regarding this point. Dubrunfaut (3), and Hönig and Rosenfeldt (6) have prepared sodium salts of lactose. These salts are formed in alkaline solutions, and that doubtless accounts for the fact that at high pHs the equilibrium rotation is reduced. According to Bleyer and Schmidt (1), the original value is restored by neutralization of the alkali. Levy and Doisy (10) also found that lactose unites with borates in solutions, though the action is not pronounced. The work of Trey (16) has already been mentioned.

SUMMARY

In glycerol solutions, the equilibrium mixture of the high and low rotating forms of lactose contains more of the high rotating component than is found in aqueous solutions.

The specific rotation of lactose is increased in glycerol solutions but, according to other workers, it is decreased in alcoholic or acetone solutions.

Therefore, it may be assumed that water is not an important factor in determining the ratio of the sugars at equilibrium.

This implies that the amount of aldehydrol present in solution is small, or else that its specific rotation is approximately equal to the weighted mean of the rotations of the two anhydrides, taking their equilibrium concentrations into consideration.

The specific rotation of lactose is altered by the presence of salts. The effect is small in dilute solutions.

Changes in the concentration of lactose, or of the salt, result in shifts of the equilibrium rotation which are in agreement with the theory that molecular compounds are formed in salt solutions.

REFERENCES

- (1) BLEYER, B., AND SCHMIDT, H. Studien über das Verhalten der wichtigsten Kohlenhydrate in stark saur alkalischer sulfid und bisulfithaltiger Lösung. III. Einwirkung von Alkalien auf der Kohlenhydrate. *Biochem. Z.* **141**: 278-96. 1923.
- (2) BROWNE, C. A. A Handbook of Sugar Analysis. 1st edition, p. 25. John Wiley and Sons, New York. 1912.
- (3) DUBRUNFAUT. Note sur le sucre de lait. *Compt. rend.* **42**: 228-33. 1856.
- (4) GILLIS, J. Nouvelles recherches sur le sucre de lait. *Rec. trav. chim.* **39**: 88-125. 1920.
- (5) HOLTY, JOSEPH GERARD. Solubility and specific rotatory power of carbohydrates and certain organic acids and bases in pyridine and other solvents. *J. Phys. Chem.* **9**: 764-79. 1905.
- (6) HÖNIG, M., AND ROSENFELD, M. Zur Kenntniss einiger Zuckerarten. *Ber.* **12**: 45-7. 1879.
- (7) HUDSON, C. S. Über die Multirotation des Milchezuckers. *Z. physik. Chem.* **44**: 487-94. 1903.
- (8) HUDSON, C. S. The hydration of milk sugar in solution. *J. Am. Chem. Soc.* **26**: 1065-82. 1904.
- (9) HUDSON, C. S. A review of discoveries on the mutarotation of sugars. *J. Am. Chem. Soc.* **32**: 889-94. 1910.
- (10) LEVY, MILTON, AND DOISY, EDWARD A. The reaction of borate and sugars. II. The optical activity of sugars in borax solutions and the configuration of mutarotatory isomers. *J. Biol. Chem.* **84**: 749-62. 1929.
- (11) PUCHER, GEORGE, AND DEHN, W. M. Solubilities in mixtures of two compounds. *J. Am. Chem. Soc.* **43**: 1753-58. 1921.
- (12) TANRET, C. Sur les modifications moleculaires du glucose. *Bull. soc. chim.* (3) **13**: 728-35. 1895.
- (13) TOLLENS, B. Ueber die spezifische Drehung des Rohrzuckers in verschiedenen Lösungsmittel. *Ber.* **13**: 2297-2302. 1880.
- (14) TREY, HEINRICH. Experimentalbeitrag zur Birotation der Glykose. *Z. physik. Chem.* **18**: 193-218. 1895.
- (15) TREY, HEINRICH. Ein weiter Beitrag zur Birotation der Glycose. *Z. physik. Chem.* **22**: 424-63. 1897.
- (16) TREY, HEINRICH. Ein Beitrag zu den Rotationserscheinungen der Laktose. *Z. physik. Chem.* **46**: 620-719. 1903.
- (17) U. S. Bureau of Standards. Polarimetry. U. S. Bureau of Standards, Circular No. 44: p. 150. 1917.

BIRTH-WEIGHT, GESTATION PERIOD, AND SEX RATIO OF ALASKAN HYBRID HOLSTEIN-GALLOWAY CALVES

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The birth-weight, gestation period, and proportion of female calves are of general interest to all dairy cattle breeders. There is included herewith data recorded by the author, upon the length of gestation, birth-weight, and a ratio of sexes for hybrid, Holstein-Galloway cattle of the Alaska Agricultural Experiment Stations during the period of 1917-1932.

The purpose of the original experiment, from which the data were taken, was that of producing a hardy dairy cow for Alaska. The work had progressed to point where a number of hybrid cows were producing more than 10,000 pounds of milk and 400 pounds of butterfat per lactation. The project, supported by Department of Agriculture appropriation, was abandoned July 1, 1932, and the supervision of the work turned over to the Alaska Agricultural College and School of Mines.

The original breeding experiment was begun by making fifteen matings between registered Holstein bulls and registered Galloway cows. Later, ten reciprocal matings were made between the Galloway bulls and Holstein cows, as well as a number of backcrosses, when a limited number of hybrid cows were mated to Holstein or Galloway bulls. (Tables 1 and 1a.)

GESTATION PERIOD

The number of days from date of breeding to parturition was recorded for the Holstein-Galloway hybrids. Both breeding and parturition dates were included in the period of gestation calculations.

There appears to be no significant difference in the length of gestation between the hybrid females and the hybrid males of this herd. A mean of 282.9 ± 3.2 days was found to be the average period of gestation for the 116 hybrid calves. Fifty-five were males, which were carried by their dams for an average gestation of 282.8 ± 3.4 days, while 61 females were carried an average of 283.1 ± 3.0 days. (Figure 1.) Thirty of the 55 males were carried by their dams between 279 to 286 days. Ten of the 55 males, 18.1 per cent, were carried 279 days or less. Fifteen, 27.2 per cent, were carried 286 days or more. Forty-five of the females were carried by their dams from 280 days to 286 days. Five of the 61 females, 8.3 per cent, were carried 279 days or less, while 11, 18.0 per cent, were carried 286 days or more. The shortest gestation was for each of two hybrid males, a period of 269 days each. The shortest hybrid female gestation was 270

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TABLE 1
Galloway and Holstein parent cattle

(a) GALLOWAY CATTLE		
Alaskan herd no.	Name	Reg. no.
<i>Cows</i>		
10	Mollie C. of Red Cloud	25239
71	Mollie 3rd of Kodiak	34911
123	Miss 2nd of Kodiak	40338
140	Bertha A	34889
147	Hattie B	32416
187	Fidelia 4th of Kodiak	40335
209	Lady May of Kodiak	41738
215	Fidelia 6th of Kodiak	41730
246	Maggie of Kodiak	45143
247	True Beauty 3rd of Kodiak	45138
252	Mollie 8th of Kodiak	45146
277	Jaynes Banshee	48193
293	Miss of Red Cloud's Girl	48148
<i>Bulls</i>		
163	Carnot of Kodiak	39139
218	Prince Douglas 3rd of Kodiak	45136
303	Ranger of Seven Oaks	45044
329	Aberdeen	2646*
(b) HOLSTEIN CATTLE		
<i>Cows</i>		
2H	Cascade Betsy	154663
3H	Cascade Betsy 2nd	260870
4H	Miss Gladys Cornucopia	336455
5H	Grandview Fayne Picbe Johana	233446
6H	Gladys Mercedes Banks	336456
9H	Gladys Mercedes Banks 2nd	596282
13H	Kodiak Mercedes Banks	596283
16H	Betsy Chinacum Cornucopia	692342
25H	Miss Gladys Shadford	963107
<i>Bulls</i>		
1H	Chinacum Sir Quirinus Cornucopia	176468
7H	Islander (Eligible to registry)	
20H	Shadford Segis Hartog 2nd	352564
35H	Heilo Pontiac King	519811

* Canadian herd book registry

days. The longest gestation for a male was 293 days, and the longest period of gestation for a female was 299 days.

The reports of several investigators indicate that there is a variation in the gestation period for the different breeds of cattle. Wing (1912) reported that from a mixed herd of Jersey and Holstein calves, 182 calves averaged 280 days of gestation. The shortest was 264 days and the longest 292 days, and the period was approximately the same for both sexes. Wellman (1910) reported 288 Hungarian cows carried their calves an average of 284.61 days and 291 Simmenthal cows carried their calves for an average of 291.2 days.

TABLE 1a
Twenty-five reciprocal Galloway and Holstein Matings

SIRE P ₁	DAM P ₁	OFFSPRING F ₁
<i>Galloway</i>	<i>Holstein</i>	
Herd No.	Herd No.	
163	5H	♀ 5
218	5H	♂ 8
163	3H	♂ 6
303	6H	♀ 14
303	9H	♂ 15
303	2H	♂ 22
303	4H	♀ 30
303	13H	♂ 40
329	25H	♀ 52
329	16H	♂ 66
<i>Holstein</i>	<i>Galloway</i>	
Herd No.	Herd No.	Herd No.
1H	71	♀ 1
1H	147	♂ 2
1H	187	♀ 3
1H	10	♀ 4
1H	246	♂ 7
1H	247	♂ 11
1H	277	♀ 16
1H	209	♂ 17
1H	293	♀ 18
1H	215	♂ 19
1H	123	♂ 20
1H	140	♀ 21
20H	215	♀ 28
1H	252	♂ 33
20H	246	♀ 39

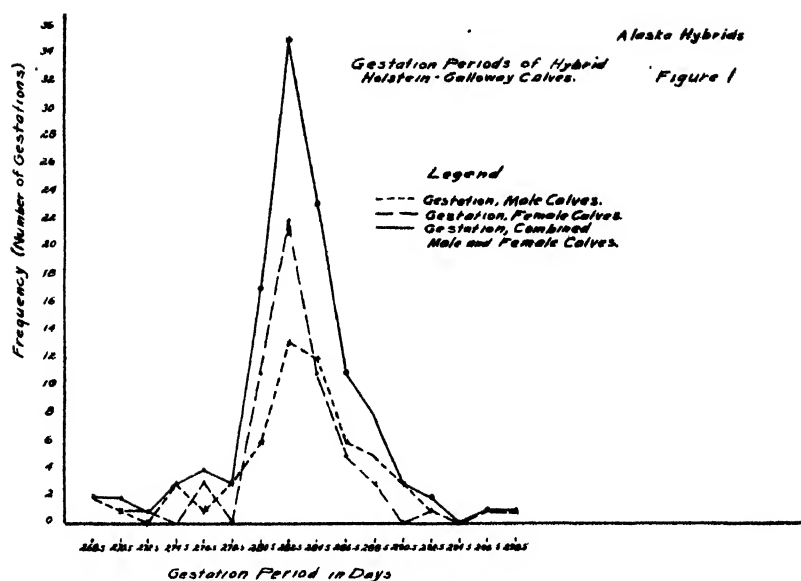
♀ Female.

♂ Male.

McCandlish (1922) in a summary based upon 369 gestations found a mean of 280 days in the dairy herd at the Iowa State College. McCandlish decided that the length of gestation was not affected by sex of the calf, breed or age of the cow, or season of freshening. Contrary to McCandlish's findings, Fitch, McGilliard, and Drumm (1924) found a difference in length of gestation between the breeds at the Kansas Station as follows:

Average gestation for Jerseys	284.3 days
Average gestation for Guernseys	283.0 days
Average gestation for Ayrshires	284.6 days
Average gestation for Holsteins	281.0 days

Copeland (1930) studying Jersey cattle, found an average gestation of 278.5 days for 1,075 Jersey cows. His data showed that males were carried an average of one day longer than females. Hooper and Nutter (1924) reported that 59 per cent of the calves dropped by 44 cows that carried their calves more than 280 days were males.



The data obtained upon the average length of gestation for the Alaska hybrids agree closely with the generally accepted average of 283 days for cattle. (Henry and Morrison, 1923, and Dawson, 1925.) No significant difference was noted between the gestation periods for the calves of the first four generations of hybrids. The one and only male of the fifth generation was carried in gestation 287 days (Table 2).

TABLE 2
Gestation period of Holstein-Galloway hybrids

SEX AND GENERATION	AVERAGE GESTATION PERIOD (DAYS)
13, F ₁ hybrid males	284.7
12, F ₁ hybrid females	283.9
21, F ₂ hybrid males	281.2
19, F ₂ hybrid females	282.5
16, F ₂ hybrid males	281.8
24, F ₂ hybrid females	283.2
4, F ₄ hybrid males	284.2
6, F ₄ hybrid females	286.6
1, F ₅ hybrid males	287.0

BIRTH-WEIGHT OF HYBRID GALLOWAY-HOLSTEIN CALVES

The birth-weight of calves has been reported upon by several investigators. Dvorachek and Semple (1931) report an average birth-weight of 64.2 pounds for 21 Arkansas native calves, and 66.9 pounds for 21 purebred Aberdeen Angus calves, and 61.5 pounds for 20 crossbred calves.

When the first crossbred females were backcrossed to purebred Angus, 23 hybrid calves were produced which weighed 65.9 pounds.

In a study of a number of breeds, Eckles (1919) records the birth-weights of the University of Missouri herd calves as follows:

BREED	NO. CALVES BOTH SEXES	AVERAGE WEIGHT
Jerseys	196	55.0 lbs.
Holsteins	154	90.0 lbs.
Ayrshires	53	69.0 lbs.
Dairy Shorthorns	30	73.0 lbs.

From miscellaneous data compiled by Eckles:

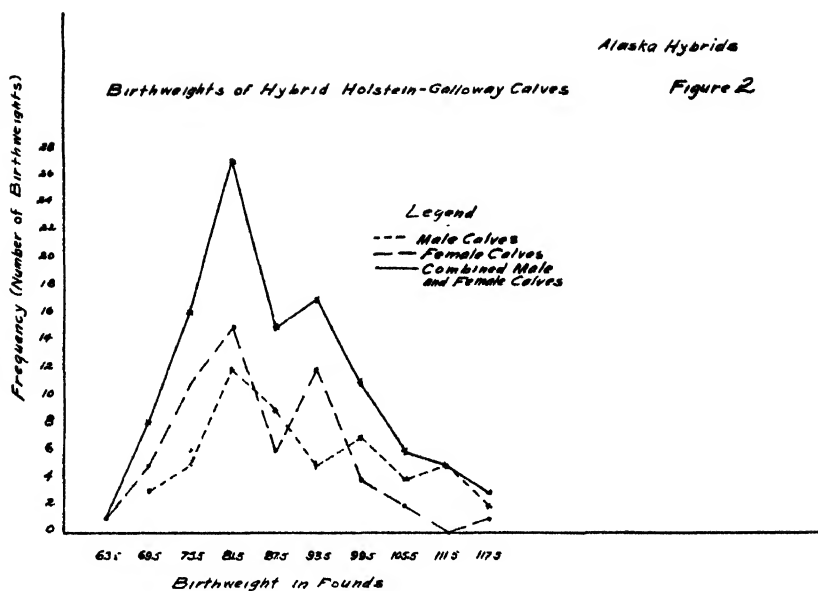
BREED	NO. CALVES BOTH SEXES	AVERAGE WEIGHT
Jerseys	253	55.0 lbs.
Holsteins	229	89.0 lbs.
Guernseys	57	57.0 lbs.
Ayrshires	80	76.0 lbs.
Brown Swiss	5	100.0 lbs.

Hulse and Nevens (1917) report considerably heavier average birth-weights for Jersey and Guernsey calves, and approximately the same weight for Holstein and Ayrshire calves; the weights were 88 pounds for female Holstein calves and 90 pounds for male Holstein calves. The number of individuals was not given.

Comparing the average birth-weights of the Alaska Agricultural Experiment Station hybrids, 87.9 ± 8.2 pounds, with the average birth-weights given by Eckles and Hulse and Nevens, it appears that Holstein calves average slightly heavier at birth than the Galloway-Holstein hybrids. Eckles states that cows between the ages of five and ten years produce the maximum sized calves. Most of the calves studied in the Alaska experiment were from dams three to six years of age.

Birth-weights were obtained for 109 of the 116 hybrids. The average birth-weight for the 109 hybrids was 87.9 ± 8.2 pounds (fig. 2). The range of the probable error indicates that the birth-weights of half of the calves were between 78.6 and 96.1 pounds. Fifty-two were males averaging 91.0 ± 8.7 pounds, and 57 were females averaging 85.0 ± 7.2 pounds. There seems to be no significant difference in birth-weight between the males and females of this investigation.

The birth-weights of 106 individuals were correlated with their gestation periods. These showed a positive correlation of 0.183, which is taken to indicate that for the calves of this experiment there exists little or no relationship between weight at birth and number of days carried in gestation.



The average birth-weights of each of the four generations were as follows:

21 F_1 hybrids, both sexes averaged 87.7 lbs. at birth

36 F_2 hybrids, both sexes averaged 82.4 lbs. at birth

38 F_3 hybrids, both sexes averaged 90.6 lbs. at birth

10 F_4 hybrids, both sexes averaged 85.6 lbs. at birth

Forty-eight individuals, 47.9 per cent of which were males, progeny of hybrid F_1 bulls (Nos. 6, 33, and 66) and hybrid cows, averaged 85.7 pounds at birth. Thirty individuals of which 46.6 per cent were males, progeny of the F_2 bulls (Nos. 46 and 69) and hybrid cows, averaged 94.0 pounds at birth. The figures indicate a possible increase in the birth-weight of progeny of hybrid F_2 bulls over the birth-weight of the progeny of hybrid F_1 bulls. Further investigation, however, is necessary to establish the point.

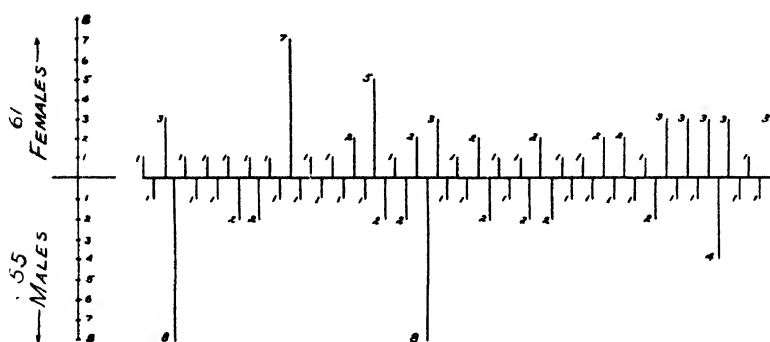
PROPORTION OF THE SEXES

Sixty-one head, 52.1 per cent, of the 116 hybrids thus far obtained have been females. For the relatively small number of animals the percentage agrees rather closely with findings of other investigators that the proportion of males to females in a population is approximately 1:1 (Wright, 1920). The ratio is due to chance sorting when a large number of animals is considered. Figure 3 shows in graphic form, the order of occurrence of the males and females for the 116 hybrids being studied. From this graph it appears that sex is due to chance sorting (Rommel, 1920). A preponderance of either sex might be shown by taking an accounting at a particular

ALASKA HYBRIDS

ORDER OF BIRTH BY SEXES FOR HYBRID HOLSTEIN-GALLOWAY CALVES.

Figure 3



time. In the first 24 births, 15 were females and 9 were ~~f~~emales, while if the first 52 animals are considered, 24 were males and 28 were females. A tabulation of the sex of the progeny of Holstein, Galloway, and hybrid bulls, is shown in Table 3. For the numbers dealt with it is believed that no significance should be attached to the preponderance of one sex or the other as they are related to individuals in Table 3. No particular signifi-

TABLE 3
Sex of progeny of bulls, showing totals for breeds and individuals

BULL HERD NO.	SIRE	DAM	OFFSPRING	
			Male	Female
1H	Holstein	13 Galloway	7	6
7H	Holstein	1 Galloway	1	0
20H	Holstein	8 Galloway	2	6
35H	Holstein	1 Galloway	1	0
			12	12
163	Galloway	2 Holstein	1	1
218	Galloway	1 Holstein	1	0
303	Galloway	7 Holstein	5	2
329	Galloway	2 Holstein	1	1
			8	4
6GH	F ₁ Hybrid	9 Hybrid	3	6
33GH	F ₁ Hybrid	32 Hybrid	18	14
66GH	F ₁ Hybrid	8 Hybrid	2	6
46GH	F ₂ Hybrid	28 Hybrid	10	18
69GH	F ₂ Hybrid	4 Hybrid	3	1
			36	45
			55	61

cance can be attached to the excessive number of females in the relatively small number of animals in this experiment, due to breed, or to individual bulls. This is of interest in view of the assertion often made by laymen and others relative to the effect upon the sex ratio of calves due to age, breed, or physical condition of the bulls.

SUMMARY

The average gestation period for 116 Holstein-Galloway hybrid calves was 282.9 days. There was no significant difference between the average gestation period of the females and the average gestation period of the males.

The average birth-weight for 109 hybrids composed of 52 males and 57 females was 87.9 pounds.

The males averaged 91.0 pounds at birth and the females 85.0 pounds.

The proportion of sexes for 116 Holstein-Galloway hybrids was 61 females to 55 males which is not statistically significant. The proportion of sexes varied in both directions when the ratios were determined with random numbers of the less than 116 head.

REFERENCES

- COPELAND, LYNN. Length of gestation in Jersey cows. *JOURNAL OF DAIRY SCIENCE* 13: 257-265. 1930.
- DAWSON, J. R. Care and management of dairy bulls. *Farmers' Bul.* 1412. Pp. 10-16. U. S. D. A. 1928.
- DVORACHEK, H. E., and SEMPLE, A. T. Beef producing qualities of purebred Aberdeen Angus cattle compared with Arkansas native cattle. *Arkansas Bul.* 247. Pp. 9-12. 1931.
- ECKLES, E. H. A study of the birth-weight of calves. *Univ. of Mo. Research Bul.* 35. Pp. 5-6. 1919.
- FITCH, J. B., MCGILLIARD, P. C., and DRUMM, G. M. A study of the birth-weight and gestation of dairy animals. *JOUR. OF DAIRY SCIENCE* 7: 222. 1924.
- HENRY, W. A., and MORRISON, F. B. Feeds and feeding, 18th ed. The Henry-Morrison Co., Madison, Wisconsin. P. 408. 1933.
- HOOPEE, J. J., and NUTTER, J. W. Sex of overtime calves. *Jour. Heredity* 15: No. 11. November, 1924.
- HULSE, R. S., and NEVENS, W. B. Feed and care of the dairy calf. *Ill. Sta. Circ.* 202. 13 p. 1917.
- MCCANDLISH, A. A. Studies in the growth and nutrition of dairy calves. *JOUR. OF DAIRY SCIENCE* 5: 301. 1922.
- ROMMEL, GEORGE M. Essentials of animal breeding. *Farmers' Bul.* 1167. P. 6. U.S.D.A. 1920.
- WELLMAN, O. The deviation of the length of gestation period according to the season of the year. *Landw. Jahrb.* 39: 409-428 (No. 3). 1910.
- WING, H. H. The period of gestation in cows. *Cornell Sta. Bul.* 162: 227-233. 1912.
- WRIGHT, SEWELL. Principles of livestock breeding. U.S.D.A. Bul. 905. P. 7. Revised July, 1923.

METHODS FOR TESTING FROZEN CREAM¹

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The fact that no accurate, dependable method for sampling and testing frozen cream is available has been the source of much controversy which has tended to hold back the development of this phase of the dairy industry. The common trade practice of standardizing cream prior to freezing slightly below the specified fat content of the frozen product may be explained by this lack of a dependable test. Ice cream manufacturers, who use frozen cream as a source of butterfat for standardizing mixes are particularly embarrassed when they do not know the exact fat content of the frozen cream. Some large buyers have demanded that their representatives be permitted to sample and test the cream before it is frozen. Such a procedure is obviously expensive and a suitable test would be an advantage both to the buyer and legitimate shippers. This work has been conducted to study the reliability of two methods for testing frozen cream.

Studies concerning the fat rising in cream (1) have shown that the fat rising in creams testing 30 to 40 per cent of fat is so slight that it should be possible to freeze such creams almost homogeneously with the methods commonly practiced in the industry. In other words, if the cream is completely frozen or even partially frozen within 24 hours after pasteurization or separation there should be no significant difference, due to fat rising, between the test of the sample taken from the top of the frozen cream and the test of the sample from the original unfrozen cream. On the other hand, with cream of low fat content, about 20 per cent, the rise of fat was found to be so great that it hardly seems possible for such cream to be frozen under the present system of freezing without having the fat rising to such an extent that the product would be decidedly heterogeneous.

Baldwin (2) suggested that the butterfat content of a can of frozen cream could be determined by testing a sample that was chipped out of the top of the can. He secured results indicating that cream containing more than 30 per cent butterfat tended to freeze homogeneously and such cream could be accurately analyzed for fat by taking a sample from any part of the unfrozen or frozen portion. Baldwin also suggested possibilities for testing frozen cream by weighing the sample directly into the test bottle before it melted. Inasmuch as practically all cream that is frozen com-

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mercially is reputed to contain 40 per cent or more of butterfat, these testing possibilities merit an investigation of their adaptability in the industry.

EXPERIMENTAL

Preparation of Cream

In order to secure results directly applicable to the conditions in the industry, arrangements were made with a local creamery organization to carry out the experiment with cream frozen in their plant during the regular routine of their work.

Sweet cream was gathered daily from points within 60 miles and hauled by truck to the plant where it was processed and put into cold storage the same day that it was received. Upon its arrival at the plant the cream was weighed and dumped into pasteurizing vats where it was standardized, then pasteurized at 150° F. for 30 minutes. As soon as the holding period for pasteurization was completed the cream was passed over a surface cooler which reduced the temperature to about 43° F. From the cooler it was poured directly into five-gallon tin cans (lard cans) that had been previously steamed. Forty-five pounds of cream were weighed into each can in such a way as to reduce foaming to a minimum. The cans were then covered with tin lids, wired shut, and placed in cold storage at about -24° F.

Each batch of cream consisted of 6,000 to 7,000 pounds, standardized with skim milk, to approximately 40 per cent butterfat. The titratable acidity of the cream was usually between 0.11 and 0.13 per cent.

Sampling of Original Cream

Samples were taken from eighty cans selected at random from eleven batches of the cream and used for this experiment. These samples were obtained with a dipper, directly after the cans were filled at the cooler, and placed in half-pint bottles that were sealed and put into a cold room maintained at 40° F. until the next day, when they were tested. Preliminary experiments indicated that the accuracy of the test was not reduced by the use of the dipper method of sampling as the butterfat was evenly distributed throughout the can.

The tests from each can were recorded and the data saved for comparison with the tests secured several months later from the frozen cream.

Sampling of Frozen Cream

Three sampling methods for the frozen cream were considered. The use of an auger to bore a sample out of the can of frozen cream was first suggested. This method proved impractical, because the auger tended to churn the cream sample.

The method suggested by Bird and Johnson (3) for sampling ice cream while frozen was tried on frozen cream. These workers took ice cream samples by removing the ice cream from the hardening rooms and letting

it stand at room temperature until it became sufficiently soft to permit a cork borer to penetrate it. Plugs aggregating 4.5 to 5.5 grams were taken with the cork borer and used for samples. When this method was applied to frozen cream the cork borers were found to be too weak for penetration until the frozen cream was melted to such an extent that definite water and fat separation was taken place. However, a cork borer was prepared with saw teeth filed around the bottom so that it could be worked into the frozen cream by turning it around while pressure was applied to the handle. The saw-toothed borer was quite successful for taking small samples and could be adapted to Mojonnier tests.

The sampling method which proved to be best suited for this experiment consisted of chipping fragments of frozen cream out of the top of the can with a small screw driver. There was always a thin layer of snow or frost present on top of the frozen cream and this had to be scraped aside before the sample was taken because the frost layer did not test the same as the cream. This frost came from the moisture which condensed from the air between the top of the cream and the lid of the can. Any foam which had remained on the cream when it was placed in cold storage was scraped away with the frost layer. Sufficient chips were removed from the cream to almost fill a half pint milk bottle without packing them. The sample bottles were pre-cooled so that the chips would remain frozen until they were weighed for analysis.

Testing Frozen Samples

Two methods for handling the frozen cream sample for testing were studied. In one case the sample was weighed into the cream test bottle while it was frozen. This method will be called the "frozen test" when reference is made to it. In the other case, the sample was melted and an attempt was made to pipette the melted cream in the same manner as one would handle normal cream. This method will be referred to as the "melted test."

"Frozen Test"

It was not practical to pulverize the frozen chips to the extent that they could be weighed directly into the neck of the Babcock cream test bottle. Therefore, funnels were placed in the cream test bottles before the bottles were balanced. These funnels had long round tops and resembled a 17.6 ml. pipette with the bottom cut off and the top extending into the neck of the test bottle. The chips of frozen cream were broken with a pair of dividers or forceps into pieces of such size that they would drop conveniently into the top or bulged part of the funnel. Nine grams of frozen cream were weighed into each funnel, which, incidentally, was about filled when the cream was slightly packed down. After the cream was weighed into the funnels the bottles were permitted to stand until the cream had melted to some extent, then 10 ml. of hot water (140°-160° F.), measured

in a pipette, was poured into the funnel in such a manner as to melt the cream remaining frozen and to rinse out the funnel, so that all of the butterfat was washed into the cream test bottle. Next the acid was added by pouring it through the funnel which thereby received another rinsing. Fifteen milliliters of acid were used. The funnels were then removed from the test bottles and the remainder of the cream test carried out according to the usual Babcock method. Excellent tests were secured when a reasonable amount of care was exercised in running them.

"Melted Test"

The melted test was carried out by melting the frozen chips remaining in the sample bottle. Specific difficulties were encountered in attempts to melt these samples. At room temperatures it took too long for the cream to melt to a point where it could be pipetted. Even when the cream was totally melted at room temperatures or at 40° F. there was such a distinct separation of the plasma from the lumpy, fat portion that it was almost impossible to get the two portions mixed together again without churning the fat. In fact, small lumps in the fat never disappeared to the extent that they did not interfere in transferring the cream with a pipette. Melting the cream with hot water soon proved impractical because the fat "oiled off" and could not be mixed into the cream again so that a representative sample could be secured in a pipette. Experimentation showed that the best method for melting the cream was to place the sample bottles in a water bath maintained at 90° to 110° F. and to agitate the bottle periodically in order to prevent "oiling off" of the fat and separation of the fat and separation of the plasma. Care had to be taken not to agitate the melted cream excessively for churning took place rather easily, and when the sample was once churned, accurate sampling was difficult. The melted samples were handled according and the cream was tested in the same manner as normal cream.

Thirty-nine of the cans placed in storage were tested and the results compared with the tests of the original cream before freezing. All of the tests were made in duplicate and reported as such with a few exceptions where accidents prevented.

Experimental Results

The average percentage of fat obtained from the tests performed on the 39 cans were:

Original	"Frozen"	"Melted"	Difference between duplicates		
			Original	"Frozen"	"Melted"
39.0769	38.6410	38.5820	0.128	0.467	0.767
Difference between Original and "Frozen"			Difference between Original and Melted		
- 0.419			- 0.457		

Analysis of Results

A general analysis of these data shows that the average or the mean test of the original samples was 39.0769 per cent; of the frozen samples, 38.6410 per cent; and of the melted samples, 38.5820 per cent. This would give the impression that the frozen samples and the melted samples tested less than the original samples, but before such an assumption can be made some factors that are involved must be considered. First, it was noticed that there was a definite difference between the duplicate tests of the same samples even in the original tests. Second, some of the tests of the frozen samples were higher than the original tests of the same samples. Six of the 39 cans tested by the "frozen" method did not give results within one per cent of the original test. Subsequent check tests proved that in three of these cases the larger differences were probably due to faulty technique in conducting the test. These check tests have also given evidence that the results for the three other cans were influenced by some factors related to the freezing conditions that were not involved in the test itself. Therefore, it was considered logical to omit the data for these three cans when discussing the merits of the "frozen" and "melted" tests.

In seventeen cases, the "frozen" tests gave higher results than the original tests of the same cream, and in nineteen cases the results were lower. The "melted" tests gave higher results in nineteen cases and lower results in fourteen cases.

Statistical Analysis

The means and standard error of the means of the three testing methods for 36 cans were found to be as follows:

Original test	39.0814 \pm .0150
"Frozen" test	39.1422 \pm .1359
"Melted" test	39.2928 \pm .2506

This indicates that 95 out of every 100 means of similar cream samples tested by the Babcock method may be expected to test within the range of 39.0514 per cent and 39.1114 per cent; 95 out of every 100 similar means obtained by the "frozen" method may be expected to test between 38.8904 and 39.4140 per cent; and 95 out of every 100 tested by the melted method may be expected to test between 38.7916 and 39.7940 per cent. It is evident from these results that the mean of the "frozen" test is more reliable than the mean of the "melted" test.

Similarly this was found to be true by determining the standard error of the mean of the differences in the results secured by the "frozen" and "melted" tests in comparison with the original Babcock test. The mean of the differences for the "frozen" test is 0.0875 and the standard error of the mean of the differences is \pm 0.0963. The mean of the differences for the "melted" test is 0.2728 and the standard error is \pm 0.2189. The standard deviations of these two sets of differences are 0.5781 and 1.2384 respectively.

Practical Application of Data

A practical interpretation of the statistical analysis would mean that any dealer, who is buying frozen cream which is supposed to contain approximately 40 per cent or more of butterfat, may check the butterfat content to the best advantage with the "frozen" test. The results indicate that if he tested as many as six cans by this method he could safely consider his average reliable within a range of 0.5 per cent of the Babcock test of the original cream. However, in case only a single can is tested, there is one chance out of four that the difference will exceed the 0.5 per cent range. If the buyer were to use the "melted" test for checking the butterfat content of the frozen cream, an average of duplicate tests on six cans could reasonably be considered within one per cent of the test of the original cream.

Inasmuch as the check tests that can be secured by the "frozen" method are more accurate and reliable than those obtained by the "melted" method, and inasmuch as the "frozen" method is more convenient to conduct in the laboratory, it is to be recommended as the best way to test frozen cream.

Summary and Conclusions

The reliability of two methods for testing frozen cream was studied by testing 36 cans of such cream and making a statistical analysis of the results obtained. The "frozen" test, performed by weighing frozen chips of cream from the top of the can directly into the test bottle, gave results which indicated that the average of six duplicate analyses was reliable within 0.5 per cent of the test of the original cream. The melted test, secured after melting the chips of frozen cream, gave results that were reliable within 1.0 per cent of the test of the original cream, when the average of six cans was used.

The "frozen" test is recommended for the butterfat analysis of frozen cream.

ACKNOWLEDGMENT

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REFERENCES

- (1) TRELOGAN, H. C., AND COMBS, W. B. Fat Rising in Cream. *JOURNAL OF DAIRY SCIENCE*. 1934.
- (2) BALDWIN, F. B. Frozen milk and cream investigations. Master of Science Thesis, University of Minnesota. 1932.
- (3) BIRD, E. W., AND JOHNSON, E. A. The effect of processing, handling and testing procedures on the fat content of ice cream. *Ia. Agr. Expt. Sta. Bul.* 287: 365. 1931.

CERTAIN FOAM PRODUCING SUBSTANCES OF MILK*

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One of the fundamental laws which govern the formation of foam is that of Gibbs and Thomson, which teaches that all matter diminishing the surface tension of water assembles at the surface. Siedel and Hesse (1) presented analytical data showing that the foam of skimmed milk contains more protein matter than the body of the milk from which the foam had been produced. This observation has since been confirmed by others and it may be assumed therefore that the capillary-active substances of milk are nitrogenous in character. Siedel (2), Hekma and Brouwer (3) have presented evidence indicating that the protein matter of skimmed milk foam is neither casein nor lactalbumin. Grimmer and Schwarz (4) based an investigation of the foam producing substances of milk upon analytical studies of separator slime and found that this material contained 36 per cent of casein and 64 per cent of proteinaceous matter other than casein or lactalbumin. Rahn and Sharp (5) state that this proteinaceous matter is probably identical with the foam producing material of milk which they designate as "Schaumstoff."

Observations made in these Laboratories during the past decade have focused attention on the character of the foam producing substances of milk. That such material is proteinaceous in nature, although not typical of either casein or lactalbumin, has been indicated on numerous occasions during the course of investigations not particularly pertinent to the subject. Certain caseins, lactalbumins, milk sugar liquors, and other integrated milk constituents have been noted to possess unpredictable foaming properties. These observations seemed to warrant further study of the capillary-active substances involved in milk foam. Such a study might in all probability contribute to a better understanding of such practical problems as foaming casein, creaming of milk, cream body, insolubility of milk powders, as well as other problems with which the milk industry is concerned.

EXPERIMENTAL

Numerous observations seem to support the view that the foam producing material exists in a highly dispersed state in milk. Therefore quantitative recovery presents certain difficulties; however, such material may be

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obtained in part from certain milk constituents with which it is associated, as for example, casein.

Certain commercial caseins possessing a high foaming tendency were eluted with dilute sodium chloride solution at appropriate hydrogen-ion concentrations. The eluted casein no longer possessed any substantial foaming ability whereas the elution product showed remarkable foaming properties. Approximately 100 cc. of freshly prepared product containing only a few milligrams of organic matter permitted the formation of a permanent foam volume of nearly 4 cubic feet. It is this elution product which served as a basic material for the studies reported hereinafter. The material as obtained was a clear, colorless liquid containing 0.072 per cent solids other than salts. Its nitrogen, phosphorus and sulfur content calculated to the dry, salt-free basis was 13.64 per cent, 0.72 per cent, and 0.60 per cent, respectively.

Surface tension measurements were made with a Du Noüy Precision Tensiometer (6). The results of the determinations made with the fresh material and after various treatments are shown in Table 1, from which it will be noted that the surface-activity is substantially the same as that of skimmed milk. Under the influence of heat the surface tension increased slightly but measurably. A similar increase is reported by Dahlberg and Hening (7) for skimmed milk as a consequence of pasteurization.

TABLE 1
Surface tension of the elution product of casein

TREATMENT OF SAMPLE	TOTAL SOLIDS* (MG. PER 100 CC.)	MINUTES BEFORE READING	SURFACE TENSION AT 25° (DYNES PER CM.)
None	72	2	51.6
None "	72	10	52.0
None "	72	30	52.0
None	72	129	50.2
None	72	240	50.9
Boiled	72	2	56.2
Concentrated at 50° and rediluted	72	2	54.2
Irradiated*	72	2	53.2
Diluted with water (1:5)	14	2	54.9
Diluted with water (1:5) and boiled	14	2	57.6
Diluted with water (1:100)	0.72	2	59.0
Diluted with water (1:1000)	0.072	2	63.6
Diluted with water (1:5000)	0.014	2	75.1

* Irradiated with a quartz mercury vapor lamp at 18 inches distance for 30 minutes.

The presence of substances in a colloidal state was further revealed by other general characteristics of the product. When exposed to a temperature of 23° C. or above a turbidity developed and slowly increased in intensity. When such warmed material was cooled slowly over a period of hours and allowed to remain at temperatures between 1 and 10°, the turbidity disappeared and a water clear or substantially water clear solution again resulted. Successive alternate warmings and coolings could be carried out for 3 to 4 times, providing the temperature was not raised too high, with visible evidence of increase in turbidity at the higher temperature and subsequent clearing of the solution at the lower temperature. However, upon successive changes in temperature the turbidity became more and more stable, ultimately resulting in a permanent cloudiness and settling out of a precipitate. Still further evidence of the unstable character of the material was manifested by the fact that on long standing in the absence of light and at relatively low temperatures turbidity developed and a precipitate formed. During the first 3 days the pH of the material shifted from 4.2 to 5.8 at which point it remained constant for many months. The constancy of the pH of the aged sample is in all probability due to the gradual insolubilization of a substance, the isoelectric point of which is, under the particular conditions, pH 5.8. Adjustment of the acidity of fresh samples and of slightly turbid samples to pH 5.8 yielded a flocculent precipitate further confirming the presence of a substance which has its isoelectric point at pH 5.8.

The above observations are interpreted to mean that a degree of supersaturation exists in the solution. The term "supersaturation" is used here in accordance with the hypothesis advanced by Schade (8). There seems to be a zone of abnormal retention of supersaturation. Any factor which will alter the conditions of supersaturation will furnish the impulse for a change from the supersaturated state of the colloid to a less dispersed state, resulting ultimately in the agglomeration of the material. Such impulses may be furnished by agitation, light, change in the hydrogen-ion concentration and raising of the temperature.

As stated above, 23° was found to be the temperature at which visible agglomeration became evident. This observation is significant, since curves for the foaming ability of milk and cream at various temperatures show a kink between 20 and 30° (9). Furthermore the curves reported by Rahn and Sharp (10) for the foam height of skimmed milk at various temperatures show a similar characteristic within the same temperature range. These authors raise the question whether these kinks indicate the presence of a specific foam producing material or of a stabilizing substance in the foam. In view of the properties shown by the material discussed in this paper it appears that the observations noted by other investigators may be due to the substance which exhibits a critical agglomeration at 23°.

The agglomerate, even after drying at room temperature, is of a vitreous or resinous character only slightly soluble in water. The clear solution of the original material showing no visible evidence of agglomeration, when dried in a dispersed state at room temperature, as in the drying of foam, was more readily dispersed in water, although not entirely so. However, if this dry dispersed material was compacted in the presence of even only a slight amount of moisture, it showed the same relative insolubility as the dried agglomerate. The agglomerate freed from electrolytes as far as possible was soluble in water after prolonged boiling to the extent of only 40 mg. per 100 cc. Such a solution showed a surface tension of 51.6 dynes per cm. which is equal to that of the original freshly obtained product.

In view of the generally accepted opinion that nitrogenous matter concentrates in the foam, quantitative removal of the material contributing to the formation of foam was attempted by whipping. An appropriate amount (about 500 cc.) of the original material was whipped by suitable means. The foam thus obtained was removed from the surface of the liquid with a minimum of compaction and allowed to drain on a wire screen. The remaining liquor was combined with that which had drained from the foam. The slightly turbid mixture was again whipped and the foam was removed as above. The less turbid liquid now obtained was again whipped but yielded only a very small amount of a relatively unstable foam. The liquid remaining after the removal of the foam showed no evidence of turbidity. Analytical data were obtained for the dried foams and the remaining liquids. The results are shown in table 2 from which it is to be noted that as the foam is removed the nitrogen and phosphorus content of the remaining liquid becomes less and less. The entire process approaches a quantitative removal of nitrogen and phosphorus. The liquid after practically complete removal of nitrogen and phosphorus still possessed capillary-activity and showed a surface tension of 61.9 dynes per cm.

TABLE 2

Nitrogen and phosphorus content of the elution product of casein, foam and residual liquid

DESCRIPTION OF MATERIAL	PER CENT NITROGEN ON DRY BASIS	PER CENT PHOSPHORUS ON DRY BASIS
Original	4.35	0.09
First Foam	10.93	0.31
First Liquid	2.30	0.06
Second Foam	9.19	0.22
Second Liquid	0.92	0.04
Third Liquid	0.75	Trace

The phosphorus of the original as well as of the foam samples was not true inorganic phosphorus. Other data obtained with similar foam products have always shown phosphorus in organic combination in amounts varying between about 0.2 per cent and 1.8 per cent on the dry basis. Likewise cholesterol was found to be present in all foam producing solutions according to qualitative tests. The presence of phosphorus in organic combination and of cholesterol should not be lost sight of in any technical study of the foam producing substances of milk. Sulfur has also been found in all such material and it is significant that the material contained no cystine.

Since there is ample evidence indicating an interrelationship between the substances contributing to the formation of foam, the churning of butter, viscosity, as well as other physical characteristics of cream and various milk mixtures, it seemed desirable to ascertain by simple and direct experimentation, whether the material under investigation could be shown to influence certain of these phenomena. Inasmuch as considerable speculation has been advanced concerning the factors affecting the cream layer of milk and the body of cream, the elution product with which we are concerned was added to whole milk in minute quantities for the purpose of determining its effect upon the depth of the cream layer.

Five cc. portions of the material, containing only 0.072 per cent solids other than salts, were added to 200 cc. portions of fresh fluid whole milk. After mild agitation 10 cc. portions of the mixture were transferred to graduated tubes and the cream allowed to rise during periods of 24 and 48 hours. The effect upon the cream volume is shown in the accompanying table 3. An increase in cream volume was noted in all cases. Equivalent results were obtained with other samples similarly treated, the data recorded being only a summary of numerous observations. It will be noted that the material added to sample 2 was in a more highly dispersed state than that used for the other samples and that it caused the greatest increase in cream volume. There was a reduction in effectiveness of the added material caused by heat, aging, and light. It is apparent that the increased cream volume is due primarily to the physical state in which the material exists rather than to the gross quantity added; only 1.8 mg. of active solids were mixed with 100 cc. of whole milk. Viscosity of the solution added could not have been a contributing factor, because measurements were made with a Sabolt Viscosimeter and showed values identical with that of water within a temperature range of 12 to 65°.

The instability of the material under the influence of heat, agitation, and aging was likewise manifested by exposure to ultra-violet rays. When exposed to the rays of a quartz mercury vapor lamp for sufficient length of time an odor developed resembling somewhat that of over-irradiated milk, but more particularly that which results from exposure of human skin to strong ultra-violet light. This suggests the mobilization of SH-

bodies, as observed by Wels (11). The aged material also gave off the same characteristic odor during mild agitation at room temperature even though exposed to only diffused natural light.

TABLE 3
Cream volume of milk as affected by the elution product of casein

SAM- PLE NO.	MATERIAL ADDED TO 200 CC. OF MILK	DESCRIPTION	CREAM VOLUME IN 10 CC. OF MILK AFTER		PER CENT INCREASE IN CREAM VOLUME
			24 HRS.	48 HRS.	
1	5 cc. water.	Practically clear	cc. 1.6	cc. 1.6	0
2	5 cc. elution product 24 days old, filtered.		2.4	2.6	62
3	5 cc. same as No. 2 but de- canted immediately above the precipitated agglom- erate.	Slightly turbid	2	2	25
4	5 cc. same as No. 2 but boiled.	Turbid	2	2	25
5	5 cc. same as No. 2 but irradiated.*	Slightly turbid	2	2	25

* Irradiated at 18 inches from a quartz mercury vapor lamp in 0.2 mm. layer for 15 seconds.

These observations indicate that the excitation by ultra-violet rays, by heat, or by agitation may affect the sulfur containing constituents of the material under investigation. From a strictly physical point of view such a common effect is not impossible; because heat, agitation, and radiant energy are essentially common forms of energy insofar as their ability to interfere with the Brownian movement of the molecules is concerned. This conception of the influence of these physical forces is further emphasized by various observations noted with the material, such as an odor characteristic of that of boiled milk noted during concentration at low temperature, after long standing at room temperature, or as a result of successive alternate warmings and coolings. It is generally known that during the boiling of milk or even heating to only about 85°, volatile sulfur compounds are formed (12). Analytical data have shown the presence of sulfur in appreciable amounts in the material under consideration, but the analytical proof of the migration of sulfur could not be obtained due to inadequacies of the available methods for the estimation of the minute quantities involved.

Isolation of the foam producing substances by dialysis was impossible due to extreme variations in diffusability apparently caused by the unstable character of the material. Dialysis of the fresh product permitted the recovery of only a small percentage of the nitrogenous matter, whereas still less nitrogen was retained with older samples which had apparently under-

gone a chemical change under the influence of the above mentioned physical factors.

Of the numerous attempts to quantitatively recover, by precipitation, the complex of substances, contributing to the formation of foam, that with chlorine gas proved to be the most effective. The liquor from which the precipitate formed by the use of chlorine gas had been removed showed no foaming properties whatsoever. Furthermore, such liquors contained no nitrogen, or at the most only minute traces. The precipitate, after suitable removal of excess chlorine was found, when redispersed in water, to possess physical properties and a gross chemical constitution similar to that of the original solution.

Chlorine gas can be applied in a practical way for indicating even small amounts of the foam producing material. Such material associated with milk sugar in minute quantities which cannot be detected by such precipitants as acid mercuric nitrate or phosphotungstic acid, is manifested by the production of foam and turbidity upon the introduction of chlorine gas.

The foam producing material was also quantitatively recovered by precipitation with chlorine gas from various milk sera from which the casein alone, or the casein and lactalbumin, or casein, lactalbumin and lactoglobulin had been removed. The greatest yields were obtained from the residual milk substances after the removal of the fat, casein, lactalbumin, most of the calcium and phosphorus and a very large proportion of the milk sugar. Specimen products obtained from this material have shown, when redispersed in water, in low concentration a surface tension of as low as 45 dynes per cm. The material recovered from any of the above milk sera possessed characteristics similar to those recorded for the casein elution product.

SUMMARY

The various data and observations as presented would seem to show that the particular group of substances contributing to the foaming ability of milk is contained in that group of milk constituents commonly designated as the "nitrogenous extractives." Such substances are physically associated with the better classified milk constituents, particularly the proteins. The physico-chemical properties of the highly dispersed and unstable colloids contributing to the foaming property of milk and to the character of milk foam are no doubt responsible for many other physical characteristics and reactions of milk and its derivatives. The reactions manifested by these colloids under the influence of change in hydrogen-ion concentration, salt content of the medium, temperature change, agitation, radiant energy, and aging serve as a basis for assigning to this milk fraction a significant rôle in many common phenomena with which the dairy chemist and milk technologist is familiar.

It is probable that the lipid material which is associated with the milk proteins and especially with the nitrogenous colloids discussed in this paper contributes not only to the surface-active characteristics of the material with which it is prosthetically bound, but also to the other physical properties exhibited by milk and certain of its derivatives. Direct proof of the presence of a saponin-sterol grouping as a normal constituent of the surface-active substances under consideration is difficult. However, certain evidence would seem to indicate such a possibility. The precise character of the proteinaceous constituent of the material dealt with in these studies cannot be defined in terms of the usual classifications without reservation. Lactoglobulin is strongly suggested by many of its properties; however, irregularities in chemical constitution and many of its physical reactions prevent firm conclusions to this effect.

The conception of prosthetically bound entities or specific groupings which influence or in fact may determine primary reaction characteristics of specimen products such as milk and certain of its constituents, is in conformity with considerable data of recent origin. Such a concept permits an approach to many complicated problems prevalent in the milk industry which is impossible from a strictly analytical point of view. In a previous paper from these Laboratories (13) it was shown that certain biological activities exhibited by milk constituents may be due to a prosthetically bound complex involving the milk proteins, especially the lactalbumin, and cholesterol.

In considering the various observations recorded in this paper from a purely biochemical standpoint the colloidobiological study of the vitamins by von Hahn (14) should be mentioned. The author considers vitamin activity in parallel with surface-activity. The activation and inhibition of certain enzymes may be dependent upon capillary-activity, as Glick and King (15) have recently reported. In all probability digestion, assimilation, and elimination are influenced by surface-active substances. Notwithstanding the speculative possibilities concerning the relationship between the surface-active properties of milk substances and physiological matters, it is reasonably certain that such properties exhibited by segregated milk constituents indicate their potential rôle in affecting many phenomena commonly observed in the milk industry.

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REFERENCES

- (1) SIEDEL, J., AND HESSE, A. Über das Schäumen der Milch bei der Entrahmung mittelst Centrifugen. *Molkerei-Zt.* Hildesheim. 14: 637, 654. 1900.
- (2) SEIDEL, J. Über einige den Butterungsvorgang betreffende Beobachtungen und eine hierauf begründete Erklärung der Butterbildung. *Molkerei-Zt.* Hildesheim. 16: 505, 525. 1902.
- (3) HEKMA, E., AND BROUWER, E. Over melkschuimvliesjes en de aan hunne vorming ten grondslag liggende substantie. *Verslag. landb. Onderzoek. Rijkslandbouw-proefsta.* 28: 46. 1923.
- (4) GRIMMER, W., AND SCHWARZ, G. Zur Kenntnis des Zentrifugenschlammes. *Milch-wirtschaft. Forsch.* 2: 163. 1925.
- (5) RAHN, O., AND SHARP, P. F. *Physik der Milchwirtschaft*, Berlin, 1928, p. 7.
- (6) DU NOÛY, P. L. An apparatus for measuring surface tension. *Jour. Gen. Physiol.* 1: 521. 1919.
- (7) DAHLBERG, A. C., AND HENING, J. C. Viscosity, surface tension, and whipping properties of milk and cream. *N. Y. State Agr. Exp. Sta., Bull.* 113, August, 1925.
- (8) SCHADE, H. Concretions. ALEXANDER, J., *Colloid Chemistry.* The Chem. Catalog Co., New York 2: 803. 1928.
- (9) ASSOCIATES OF ROGERS, L. A. *Fundamentals of Dairy Science.* The Chem. Catalog Co., New York, 172. 1928.
- (10) RAHN, O., AND SHARP, P. F. *Physik der Milchwirtschaft*, Berlin, 1928, p. 16.
- (11) WELS, P. Die Entstehung eines reversibel oxydierbaren Körpers durch Bestrahlung von Eiweiss. *Arch. exptl. Path. Pharmacol.* 171: 480. 1933.
- (12) PORCHER, CH. *Le lait desséché.* 2nd ed., Lyon, 1926, p. 81.
- (13) ANSBACHER, S., AND SUPPLEE, G. C. The cholesterol content and the antirachitic activation of milk constituents. *Jour. Biol. Chem.* 105: 391. 1934.
- (14) V. HAHN, F.-V. Colloidobiological study of the vitamins. ALEXANDER, J., *Colloid Chemistry.* The Chem. Catalog. Co., New York 2: 917. 1928.
- (15) GLICK, D., AND KING, C. G. Relationships between the activation of pancreatic lipase and the surface effects of the compounds involved. The mechanism of inhibition and activation. *Jour. Biol. Chem.* 97: 675. 1933.

PASTURE FERTILIZATION RESULTS

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In the fall of 1929, pasture management trials were started at the Southwestern Louisiana Institute dairy farm, Lafayette, Louisiana. The land is rolling prairie type, slightly acid and somewhat low in fertility due to continual cropping.

Section A, consisting of eight acres that had been in silage corn in 1929, was disced thoroughly and seeded to White Dutch clover and domestic rye grass in October and lespedeza added in February. Bermuda and carpet grass have come in since then. In March nitrate and superphosphate were added singly and in combination and, even in the dry season of 1930, approximately doubled the yields of hay over check plots. In late fall one-half of these eight-acre plots was limed with two tons of calcium carbonate (crushed oyster shell) per acre. Rye grass was again seeded and the same rate of fertilization followed on each plot for the following three years. One-half of the nitrate was applied in the spring and other fertilizer in the late fall. This section was cut three times, and in 1931 four times, for hay and the yield of cured hay determined from representative areas. The average yearly hay yield from unlimed plots, regardless of fertilization, was 7,528 pounds, and for limed 8,705 pounds, or 15.6 per cent greater. The average hay yield for check plots not fertilized was 6,871 pounds. Annual application of 200 pounds nitrate of soda gave 9,861 pounds of hay, or 43.5 per cent increase. Two hundred pounds of 18 per cent superphosphate gave 8,323 pounds hay, or 21.1 per cent increase; while nitrate and superphosphate together gave 10,071 pounds hay, or 46.6 per cent increase over no fertilizer. Potash apparently had no effect on yield and the use of ammonium sulphate apparently lowered the yield 1,006 pounds, or 14.6 per cent through adverse effect on white clover.

Section B, consisting of four acres of permanent pasture, was given similar treatment except grazed and yields determined from caged areas. Here the response to fertilizer was more marked, with an average increase of 65.8 per cent for nitrate of soda and 180.5 per cent for nitrate and superphosphate. Still another similar field (E) has given 103.6 per cent increase in hay for nitrate and superphosphate during the last two years. Field C, similar to A in that it was seeded in 1929, but treated with 150 pounds of a complete fertilizer and 150 pounds cyanamid each year, aver-

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aged 12,976 pounds hay, or 90.7 per cent increase over check plots for the past three years.

Every cutting of hay was analyzed and is expressed as percentage of dry matter, total dry matter, crude protein, and total digestible nutrients in Table 1. The effects of fertilization on composition is not as great as the seasonal difference between cuttings of hay. Liming apparently had little effect on composition.

TABLE 1

Average composition and yield of hay cut from fertilized pasture plots, Lafayette, Louisiana—1931-1933

DATE	NO.	TREATED	PER CENT OF DRY MATTER					POUNDS PER ACRE		
			Protein	Fat	N.F.E.	Fiber	Ash	Dry matter	Protein	T.D.N.
1st cutting	21	Limed	17.12	2.97	44.00	25.49	10.40	3116.13	504.30	1921.61
"	21	No lime	17.13	2.94	44.99	25.09	9.80	2373.36	408.03	1522.10
2nd cutting	21	Limed	13.75	2.11	43.96	31.54	9.71	1184.29	194.60	654.88
"	21	No lime	13.82	2.09	41.97	32.83	9.31	1129.92	196.72	632.93
3rd cutting	21	Limed	9.33	1.98	49.44	30.99	8.44	2046.88	142.04	994.38
"	21	No lime	8.58	2.09	49.33	31.47	8.42	1985.84	131.06	996.98
1st cutting	40*	Fertilized (N or P)	17.35	2.95	43.45	25.54	10.66	3545.24	589.89	2182.54
"	15	None	16.33	2.94	45.75	24.82	10.15	2037.00	310.89	1259.48
2nd cutting	40	Fertilized	14.21	2.08	42.85	31.24	9.80	1630.99	255.87	880.23
"	15	None	13.33	2.21	43.27	31.48	9.69	961.71	160.91	537.98
3rd cutting	37	Fertilized	9.46	1.98	48.44	30.55	9.52	2847.70	235.27	1412.15
"	14	None	8.75	2.25	49.29	31.17	8.54	2158.50	142.24	1073.01
All	61	1st cutting	16.70	2.94	44.62	25.42	10.30	2946.20	478.75	1816.50
All	61	2nd cutting	13.76	2.09	43.01	31.87	9.61	1425.39	224.25	778.50
All	57	3rd cutting	9.06	2.02	48.71	31.72	8.47	2556.09	197.90	1267.97
(1931) only	21	4th cutting	6.56	1.49	52.07	31.52	8.36	5205.30	341.80	2485.20
Season	40	Fertilized	13.78	2.35	44.82	29.07	10.01	8010.61	1090.65	4481.02
	15	None	12.90	2.47	46.03	29.11	9.48	5129.58	618.58	2863.84
Season	21	Limed	13.40	2.35	45.80	29.34	9.52	6347.40	841.04	3570.87
	21	No lime	13.18	2.37	45.43	29.78	9.18	5489.12	735.81	3152.01

* Plot 8 (ammonium sulphate) not included here.

GRAZING RESULTS

Plot B (and a check plot) was grazed for three summers, and C and E for the past two summers. As B and E were treated alike, the results are given together for an average of five trials. Milking Jersey cows fed grain in proportion to milk yield were used and added or taken off as growth of grass permitted.

The check pasture averaged 164 cow days, 3411.6 pounds 4 per cent corrected milk, and a feed replacement value of \$18.57 per acre. The pastures treated with 200 pounds nitrate of soda and 200 pounds of superphosphate averaged 292 cow days, 6697.8 pounds 4 per cent corrected milk, and a feed replacement value of \$37.76 per acre. Section C treated with a complete fertilizer and cyanamid gave an average of 359 cow-days, 9,735.2 pounds 4 per cent corrected milk and a feed replacement value of \$42.52 per acre. The net value of the fertilizer under these conditions was then \$19.19 and \$23.95 respectively per acre. However, in checking the yields of nutrients from clipped hay against the nutrients consumed in milk production and maintenance, minus that in grain fed, it was found that approximately 46 per cent of the total available was used for production in the check lot, only 36 per cent of the nitrate and superphosphate pasture and 59 per cent from Section C. This disparity in results from the two measures used emphasizes the importance of further refinement in pasture tests.

The pH value of soils from each plot has been determined each year. The trend has been gradually toward less acidity in the limed and basic fertilized plots. The limed plots averaged a pH of 6.7, while the unlimed portions of the same plots averaged a pH of 5.9 early this year (1934).

SUMMARY

Pasture vegetation under conditions of this test responded quickly and profitably to nitrate, superphosphate, and cyanamid application. It responded more slowly to liming. Potash and ammonium sulphate was not effective.

The response was in greater growth rather than effecting composition of plants. There was a large seasonal difference in protein and yield with regard to time of cutting, however.

Milk production from nitrate and phosphate treated pasture was doubled, and from cyanamid, trebled over unfertilized plots.

Soil reaction has not changed adversely through proper fertilization.

PROGRESS REPORT ON COMPARISON OF LACTATION AND YEARLY RECORDS

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There has been some discussion in recent years of the relative merits of lactation records and Cow Testing Association yearly records as a basis for selecting dairy cows. Production records from 35 herds, including more than 1800 lactation records made by 552 cows and approximately the same number of Cow Testing Association yearly records made by the same cows have been studied, in an attempt to determine which of the two kinds of records is the more accurate.

These 35 herds were selected at random from herds owned by dairymen who had been members of Iowa Cow Testing Associations for at least three years. All records of all cows were used without selection, provided the cow had been in the herd long enough to have made at least two lactation records, and at least two Cow Testing Association yearly records and provided these records appeared to be complete.

The Cow Testing Association yearly records were taken directly from the herd record books. They were computed according to the usual rules of the Association, that is, they were the production records for the Cow Testing Association year. All records were taken excepting those of heifers which freshened during the year and of cows which died or were sold after the year began. The lactation records, on the other hand, were computed to include the production from calving until the cow went dry or, in cases where the cow was in milk more than 12 months, for the first 365 days after the lactation record was started. Both kinds of records were calculated to maturity by the usual 70, 80, and 90 per cent method.

The purpose of this study was to answer the following questions:

1. Is the lactation record more accurate than the Cow Testing Association yearly record; that is, does it tend to repeat itself more closely year after year, thus making it a better criterion upon which to base the culling or selection of dairy cows?

2. If the lactation record is more accurate, is this difference in accuracy sufficient to pay for the additional labor required in its computation?

It is obvious that a record, to be useful in economic culling and for breeding selections, must be such that the future production of the cow shall be rather accurately correlated with it. In other words, in order to be effective for selection purposes, past production records of a cow must

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furnish a fairly accurate measure of what she can be expected to produce in the future. The true value of culling or selecting by the use of records is dependent, therefore, upon how closely these records tend to repeat themselves lactation after lactation or year after year as the case may be.

Comparison of these lactation records with the corresponding Cow Testing Association yearly records was made by the method called "analysis of variance" which method was introduced by R. A. Fisher in 1923, for segregating from comparable groups of data the variation traceable to different sources. "Variance" is a term used by Fisher to denote the square of the standard deviation.

As the first step in the comparison, the variance of the lactation records and the variance of the Cow Testing Association yearly records of each individual herd were analyzed independently of the other herds. Then all herds were grouped together into one large population and the variance in this whole population was analyzed for both kinds of records. In each case this total variance was analyzed into those portions attributable to different causes. For example, the variance of the entire population of records from the 35 herds was analyzed into three portions. First, there is a portion caused by influences which operated alike on all cows in the same herd but differed from herd to herd, that "between herds." Second, there is a portion which was caused by things which varied from cow to cow in the same herd but which were always the same in the different lactations of any cow, that "between cows." Finally there is a portion caused by variable conditions which made a cow vary in her production from one lactation to another or from one year to another, that "between records of the same cow."

The differences in these variances were expressed in terms of correlations which show the degree of likeness between records made by the same cow, as compared with the variation existing in the whole population of records. These correlations between records of the same cow show how nearly a cow had the same record lactation after lactation or year after year according to the two methods of figuring her yield.

In table 1 are presented the age corrected records from one herd to illustrate the method of analysis. Some cows varied quite markedly in their production records from one lactation or year to the next while other cows tended to have about the same production records lactation after lactation or year after year. For example, Mildred produced 443 pounds of fat in her first lactation and only 301 pounds of fat in her second lactation. Here is a difference of 142 pounds of fat between her two lactation records. Millie, on the other hand, produced 281 pounds of fat in her first lactation and 278 pounds in her second lactation. Here there is only three pounds difference between Millie's first and second lactation records.

TABLE 1
Age corrected records from one herd illustrating method of analysis

	LACTATION RECORDS AGE IN YEARS							C. T. A. YEARLY RECORDS AGE IN YEARS							
	2	3	4	5	6	7	8	2	3	4	5	6	7	8	9
Kitty ..					314	322	271						363	319	295
Peach ..				267	329	256						328	298	310	
Dora			338	241	228						309	231	260		
Alma					418	484	390					412	283	376	
Pansy			314	289	308							328	307	302	
Nervy			333	400								370	297		
Mabel			426	343	290					399	325	336			
Rocky				448	424						389	367			
Andy		356	306						396	353					
Frank	343	331	350					344	329	297					
Mildred	443	301						387	335						
Ring	386	278						386	291						
Kat	230	263						237	291						
Millie	281	278						281	299						

ANALYSIS OF VARIANCE				ANALYSIS OF VARIANCE			
Source	d/f	Sum of sqs.	Mean sq.	Source	d/f	Sum of sqs.	Mean sq.
Total	34	148,613	4,371	Total	34	71,753	2,110
Between cows	13	101,957	7,843	Between cows	13	41,677	3,206
Between records of the same cow	21	46,656	2,222	Between records of the same cow	21	30,076	1,432

$$\text{Deduced correlation between records of the same cow} = \frac{4,371 - 2,222}{4,371} = .492.$$

$$\text{Deduced correlation between records of the same cow} = \frac{2,110 - 1,432}{2,110} = .321.$$

If all cows had varied as much as Mildred did, the correlation between records of the same cow in this herd would have been very low, while if all the cows had been as consistent in their production as Millie then the variance between records of the same cow would have been very small and the correlation between the lactation records of the same cow would have been much larger than the correlation of .492 actually found in this herd.

It will be noted at the bottom of table 1, that the mean square or variance has been analyzed into three portions: total, that between cows, and that between records of the same cow. By subtracting the variance between records of the same cow from the total variance, that portion of the total variance which is common to all records of the same cow is obtained. This divided by the total variance gives approximately the average intra-herd correlation between records of the same cow.

The average intra-herd correlations between lactation records and between Cow Testing Association yearly records of the same cow in each of the 35 herds are presented in table 2. In the last column of table 2, the

TABLE 2
Comparison of Lactation Correlations and C. T. A. Correlations for each herd

HERD NO.	NO. OF COWS	NUMBER OF LACTATION RECORDS	NO. OF C. T. A. RECORDS	INDIVIDUAL HERD CORRELATIONS		LACTATION CORRELATION EXCELS C. T. A.
				Lact.	C. T. A.	
1	26	93	93	.353	.234	+
2	27	87	72	.436	.261	+
3	15	57	37	.551	.417	+
4	18	69	59	.154	.080	+
5	7	26	26	.357	.144	+
6	10	32	30	.018	.544	-
7	24	97	89	.608	.644	-
8	6	19	17	.699	.404	+
9	9	36	40	.310	.357	-
10	24	90	93	.143	.342	-
11	12	51	45	.287	.378	-
12	17	55	55	.528	.365	+
13	19	71	60	.470	.411	+
14	16	48	50	.522	.433	+
15	29	102	109	.352	.268	+
16	17	57	53	.246	.088	+
17	11	32	38	.044	.165	-
18	14	38	41	.182	.511	-
19	14	35	35	.492	.321	+
20	12	35	40	.669	.625	+
21	14	46	50	.438	.244	+
22	23	71	72	.418	.516	-
23	14	47	54	.034	.024	+
24	15	41	37	.412	.242	+
25	19	66	58	.256	.120	+
26	9	33	28	-.049	.214	-
27	16	59	57	.417	.190	+
28	11	34	33	.321	.129	+
29	16	58	50	.653	.348	+
30	12	36	41	.466	.054	+
31	10	27	32	.227	.088	+
32	10	34	37	.345	.363	-
33	15	44	49	.166	.223	-
34	24	80	79	.420	.287	+
35	17	71	75	.294	.357	-
	552	1877	1834			

herds in which the correlation between lactation records exceeds that between Cow Testing Association yearly records are indicated by a plus sign, while those herds in which the correlation between Cow Testing Association yearly records is greater than that between lactation records are indicated by a minus sign. It will be noted that in 23 of the 35 herds the correlation between the lactation records is the greater. The correlations between the lactation records and between the Cow Testing Association yearly records show marked variations. In general however, there is a tendency for both to be high or both to be low in any one herd.

The results obtained by grouping all the herds together into one large population of records are presented in table 3. This table shows that the correlation between random records from the same herd is 0.328 in the case of the lactation records and 0.344 in the case of the Cow Testing Associa-

TABLE 3

Analysis of variance among all lactation records and among all C. T. A. Yearly Records from the 35 herds
Lactation records not exceeding 365 days
Analysis of variance

VARIANCE DUE TO	D/F	SUM OF SQS.	MEAN SQ.	DEDUCED CORR.
All causes	1876	20,022,132	10,673	$\frac{10,673-7,168}{10,673} = .328^*$
Between farms	34	6,817,830	200,524	
Remainder	1842	13,204,302	7,168	$\frac{10,673-4,838}{10,673} = .547^{**}$
Between cows on the same farm	517	6,795,599	13,144	$\frac{7,168-4,838}{7,168} = .325^{***}$
Between records of the same cow	1325	6,408,703	4,838	

<i>C. T. A. yearly records</i>				
VARIANCE DUE TO	D/F	SUM OF SQS.	MEAN SQ.	DEDUCED CORR.
All causes	1833	17,469,119	9,530	$\frac{9,530-6,251}{9,530} = .344^*$
Between farms	34	6,224,387	183,070	
Remainder	1799	11,244,732	6,251	$\frac{9,530-4,385}{9,530} = .540^{**}$
Between cows on the same farm	517	5,623,602	10,877	$\frac{6,251-4,385}{6,251} = .299^{***}$
Between records of the same cow	1282	5,621,130	4,385	

* Correlation between random records from the same herd.

** Correlation between records of the same cow, all cows being considered as in a single population.

*** Average intra-herd correlation between records of the same cow.

tion yearly records. This degree of likeness between records merely because they were made in the same herd indicates a very significant herd effect. Such an effect results from two major causes. First, the cows in a given herd are, for the most part, subjected to as nearly the same conditions of feeding and management as possible, and second, the inherent productive ability of the cows in a given herd is apt to be more nearly the same than one would expect to find in a random sample of cows from many herds. A herd effect as large as the one noted in these data may be the main explanation for the generally observed fact that the daughter-dam correlation when the sire is held constant, is generally much lower than when computed for a whole population which includes the daughters of many sires from many herds. Many other things besides the sire are "held constant" in such a computation. This large herd effect is also a strong argument for considering in all cullings and selections, how far a record is above or below the average of the herd in which it was made, in addition to considering the absolute size of the record. However, the fact that the correlations 0.328 for lactation records and 0.344 for Cow Testing Association yearly records are so nearly alike indicates that the likeness between records because they

were made in the same herd is almost identical either way the records were figured.

The correlation between records of the same cow, cows from many herds being included in one large population of records, (0.547 for lactation records and 0.540 for Cow Testing Association yearly records), includes the resemblance between the records of a given cow which result not only from the cow's intrinsic productivity but also from the fact that all of each cow's records were made in the same herd, and therefore under much the same peculiarities of management and environment. These two correlations (0.540 and 0.547) are so nearly the same that there is practically no choice between the two as to which is the best measure of a cow's future production.

The average intra-herd correlation between records of the same cow, 0.325 for lactation records and 0.299 for Cow Testing Association yearly records, is an expression of the degree of likeness between records of the individual cow due to her inherent ability to produce. In other words, any peculiarity in the management and environment in a given herd would not affect this correlation insofar as it applied to all records made in that herd. This, therefore, is the best measure of how nearly a cow tends to repeat her production in the same herd lactation after lactation or year after year. Again there is very little difference between the correlation computed from lactation records and the one computed from Cow Testing Association yearly records. The difference that does exist between these two correlations indicates that the lactation records repeats itself a little more closely than the Cow Testing Association yearly record. However, this difference is not at all significant statistically, which means that a difference as large as this might often be found between two such samples of data taken from the very same population.

CONCLUSIONS

From the results of this study it may be concluded that the Cow Testing Association yearly record tends to repeat itself in succeeding years about as closely as the lactation record does. Therefore a general change to the lactation basis for selecting dairy cows, proving dairy sires, etc., will not lead to any material increase in accuracy. Such a change would be hard to justify if it entails any appreciable amount of additional expense or trouble.

Above all, one should never lose sight of the fact that the conditions under which the records were made are important and allowances for them should be made wherever possible. The individual owner can do this to some extent, but the man who has only the production records with no description of the conditions under which they were made can not even begin to make such allowances.

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AMERICAN DAIRY SCIENCE ANNOUNCEMENTS

LENGTH OF MANUSCRIPTS

The Board of Directors at its last annual meeting passed the following resolution which was approved by vote of the membership attending the business session in Ithaca on June 27:

“*Resolved*, that the Editor limit all articles to 12 text pages except for articles of unusual merit.”

In addition, and in view of the fact that many colleges and Experiment Stations are now buying space in other Journals, the JOURNAL will accept meritorious articles of extra length on the condition that papers in excess of 12 text pages (about 16 double-spaced typewritten pages) will be paid for at the rate of \$4.00 per text page.

Authors May Secure Cuts for Illustrations

Cuts for all graphs and pictures of articles published in the JOURNAL may be secured free by authors who request them from The Science Press Printing Company. The first of each year the old cuts on hand will be destroyed.

Engineering Papers for Next Year's Program

The following statement has been received from L. S. Palmer, chairman of the Program Committee, regarding dairy engineering papers for the annual meeting which will be held next year at the University of Minnesota:

“The program committee for the 1935 meeting of the American Dairy Science Association announces that members of the Association interested in dairy engineering and who desire that this subject be given special consideration at the next meeting may be assured that worthy papers in this field which are submitted for the program will either be given a special place in the manufacturing or production sections, depending upon the topics, or if sufficient interest is shown, will be arranged in the form of a special symposium.”

JOURNAL OF DAIRY SCIENCE

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DETECTION OF LACTIC ACID IN MILK AND CREAM

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Lactic acid is usually the principal acid produced when milk sours. For this reason, in America the titratable acidity of milk is often expressed as lactic acid. The increase in acidity due to souring is called "developed" or "real" acidity, as contrasted to the "original" acidity of the fresh milk. It has been stated many times in the literature that fresh milk contains no lactic acid. Our results indicate that if fresh milk contains lactic acid, the amount present is not over about 0.002 per cent. A qualitative test showing the presence of more than this amount would indicate souring. A quantitative determination of lactic acid would serve as an indication of the degree of souring.

The titratable acidity of fresh milk usually falls between the limits of 0.12 and 0.20 per cent, although extreme variations ranging from 0.05 to 0.50 have been reported (2). The great variation in the original acidity shows the unreliability of the titratable acidity as an indication of the presence of small amounts of developed acidity in unknown samples. Sharp and McInerney (2) have shown that by taking advantage of the relationship between the pH and titratable acidity, and the influence of developed acidity and neutralization on this relation, an indication of the developed acidity of the milk can be gained. This method serves if the developed acidity amounts to a few hundredths per cent. The bacterial count is also useful as an indication of small amounts of acidity in raw milk, but is unreliable in the case of pasteurized milk and cream.

Controversies between buyer and seller constantly arise as to the freshness of dairy products when the titratable acidity is higher than average but within the normal range, or when neutralization of the developed acidity is suspected. These disputes could be settled by a direct determination of lactic acid in the product. A direct determination of lactic acid in cream would serve as a valuable indicator of its quality, particularly if it is to be frozen and stored, since cream may have been neutralized and pasteurized. While a small amount of lactic acid in some of these products probably does no harm, it is the other associated bacteriological changes

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which may be harmful, and the presence of lactic acid serves as an indication that such changes have occurred.

Methods used for the determination of lactic acid after the removal of interfering substances may be classified as follows:

1. Precipitation of the lactic acid as an insoluble salt; for example, as zinc lactate (quantitative).
2. Oxidation of lactic acid to some substance more readily determined.
 - A. Oxidation to acetaldehyde and detection of acetaldehyde by
 - a. Color test.
 - b. Sulfite binding on distilling (quantitative).
 - c. Formation of iodoform on distilling (quantitative).
 - B. Determining CO_2 formed in partial oxidation (quantitative).
 - C. Oxidation to oxalic acid and determining oxalate (quantitative).

The oxidation of lactic acid to acetaldehyde, and the qualitative detection of the acetaldehyde by color reactions and its quantitative determination by sulfite binding, seemed to be the most promising procedures. For this reason the investigations were restricted to a study of these methods.

A later article will describe the quantitative procedure. The principal difficulty arose in the separation of lactic acid from the interfering substances which are present in milk, without recourse to complicated procedures.

PROCEDURE

Place 125 cc. of milk (more if cream is used) in an Erlenmeyer flask. Add 1.3 grams of ammonium sulfate for each 2 cc. of water in the product. (The water content need be known only approximately.) Heat the mixture to 70°C ., occasionally shaking vigorously to bring all of the ammonium sulfate into solution. Cool to below 20°C . and filter through a folded filter, refiltering the first portion that passes through the filter. Place 75 cc. of the filtrate in a separatory funnel, add 4 cc. of 5 normal sulfuric acid and 75 cc. of ethyl ether, stopper the funnel, and shake vigorously for 2 minutes. (Much of the ether used in the dairy industry for the gravimetric fat determination is not sufficiently pure, but it may be rendered satisfactory by shaking with water and distilling from an excess of water.) Allow the funnel to stand upright until the ether separates into a clear layer, and then draw off the water portion. Again shake, allow to stand, and draw off the additional water which settles. A third shaking and settling is a good practice.

Remove the stopper, and with a clean, dry cloth wipe the mouth and inside of the neck of the funnel clean and dry. Pour the ether into a glass-stoppered flask, using great care not to allow a single drop of the water phase to flow out with the ether. Add about 0.05 grams of sodium bicar-

bonate to the ether in the flask, and shake vigorously. Pour the ether into an evaporating dish, and rinse the flask into the dish with 1 or 2 cc. of water. Evaporate the ether by setting the dish on warm water. Evaporate the watery liquid to dryness over a boiling water bath. Twice rinse the dish and residue with petroleum ether, using 10 cc. for each rinsing. Warm the dish on hot water each time before discarding the ether.

Place 1 cc. of water in the evaporating dish, and cause it to flow around in the dish to dissolve the residue. Pour the solution into a test tube and rinse the dish twice into the tube, using about 0.5 cc. of water for each rinsing. Carefully evaporate the water from the test tube by holding it in a flame while shaking to avoid spattering. Stop the evaporation when about 0.25 cc. remains. Cool and add 2.5 cc. of concentrated sulfuric acid (sp. gr. 1.84). Place the tube in boiling water for 1.75 minutes, cool, and add one drop of guaiacol reagent (10 per cent of guaiacol in aldehyde-free ethyl alcohol).

A bright purplish-red color developing in a few minutes, which grows to a more intense and darker red on long standing, shows the presence of lactic acid in the milk.

This color test for lactic acid was described by Denigès (1). To gain experience and confidence, a sample of milk known to be fresh should be divided into three parts. To the first part add enough lactic acid to make 0.01 per cent, to the second enough to make 0.002 per cent, and add none to the third. Carry out the test for lactic acid on the three parts.

DISCUSSION

Before obtaining from milk an extract suitable for the application of Denigès' test, we tried many reagents for the precipitation of the proteins, but the filtrates obtained, as well as the original milk, when vigorously shaken with the ether formed emulsions which did not break even on long standing. Finally ammonium sulfate proved to be satisfactory, the emulsions obtained with the filtrates from milk and cream breaking within a few minutes, and the emulsions obtained with the filtrates from manufactured products, such as condensed milk, dried milk, and ice cream, breaking in from 15 to 45 minutes. Ammonium sulfate has other advantages, in that it increases the density of the water phase, causing more rapid settling; and it changes the water-ether distribution coefficient of lactic acid from about 10:1 to about 5:1, thereby increasing the amount of lactic acid which passes to the ether. Several other immiscible liquids were tried, but none gave as satisfactory results as ethyl ether. The petroleum ether extraction of the residue in the evaporating dish serves to remove a trace of fatty material which is not retained on the filter. When carried out with sufficient care, a recognizable color difference between a blank on fresh milk and fresh milk to which has been added 0.002 per cent of lactic acid can

be detected. A very distinct color is obtained with 0.01 per cent. Several other substances have been used to produce a color with acetaldehyde. A number of these were tried, but guiacol appeared to be the most satisfactory.

The possibility of developing this procedure into a quantitative colorimetric method was considered, but it appeared that the variables might be too difficult to control in a satisfactorily quantitative manner. Some idea of the amount of lactic acid present can be obtained, however, from the intensity of the color. The amounts of milk, milk filtrate, and ether can be reduced proportionately if less sensitivity is desired. The greatest liability of error is due to the carry over of drops of the water phase in pouring the ether from the separatory funnel. Proteins, fat, and lactose give color reactions if present in the test tube.

This method is applicable to milk, cream, dried milk, condensed milk, evaporated milk, and simple vanilla ice cream, whether neutralized or not. It is not applicable to chocolate ice cream, and possibly some of the fruits and other flavoring materials would contain ether-soluble materials which might interfere.

SUMMARY

The steps involved are saturation with ammonium sulfate, filtering, shaking the filtrate with ethyl ether, separation of the ether layer, neutralization of the acid with alkali, evaporation of the ether, washing of the ethyl ether residue with petroleum ether, taking up the residue with water, heating with sulfuric acid, cooling, and adding guiacol (Denigès test). A red color indicates lactic acid. With care the test is sensitive down to about 0.002 per cent of lactic acid in the milk.

REFERENCES

- (1) DENIGÈS, G. Réactions très sensibles des acides lactique et glycolique. *Bul. Soc. Chim. de France*. 4 ser. Vol. 5, p. 647-649. 1909.
- (2) SHARP, P. F., AND MCINERNEY, T. J. The relation of the hydrogen ion concentration to the titratable acidity of milk. *Jour. Biol. Chem.* Vol. 75, pp. 177-184. Neutralization discussed in Sixteenth Ann. Report International Assoc. Dairy and Milk Inspectors. Pp. 257-264. 1927.

THE NUTRITIONAL VALUE OF MILKS—RAW *vs.* PASTEURIZED AND SUMMER *vs.* WINTER

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The consumption of milk by both children and adults has increased in recent years. Along with this increase in the use of milk has come a greater reliance upon pasteurization as a safeguard against milk-borne diseases. It has been estimated (1) that in the United States over 87.5 per cent of the milk used in cities of 10,000 population and over is pasteurized.

Studies on the changes produced in milk by pasteurization are as old as the process itself. The question of whether pasteurized milk is equal nutritionally to raw milk is far from settled at the present time, and each new investigation seems to increase the discordance in results. Some of the reported work is valueless because it was sponsored by parties either opposed to or in favor of pasteurized milk. It is impossible to attempt a review of the existing literature on the subject in this short paper. A comprehensive review has been published recently by Stirling and Blackwood (2) of the Hannah Dairy Research Institute.

Theoretically it should be very simple to measure the biological value of all the nutrients in pasteurized milk and compare these values with those obtained with raw milk. In practice we find this method very difficult because we are probably not aware of all the nutrient factors in milk and because assay of some of the known factors is far from easy. The study of raw and pasteurized milks would be greatly simplified if the milk could be used as the sole source of the majority of the nutrients. This is now possible due to the fact that milk can be mineralized with iron, copper, and manganese and thereby rendered complete for normal animal growth. Kemmerer, Elvehjem, Hart, and Fargo (3) showed that rats reared from weaning on whole cow's milk mineralized with iron, copper, and manganese grew from 60–200 gm. in 36 days, a growth which is comparable in every way with the growth of rats on a natural ration.

Krauss, Erb and Washburn (4) found no difference in the rate of growth of rats fed raw and pasteurized milk supplemented with iron and copper. However, their animals showed a very slow rate of growth due to the absence of manganese and this slow growth may have reduced the

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requirements sufficiently to prevent the demonstration of any limitations in the treated milk.

In this paper we wish to present results obtained by feeding mineralized raw and pasteurized milk produced during the fall, winter, and spring months. We want to emphasize that the method which we have used does not give the quantitative destruction or inactivation of any individual component due to the heat, but it does demonstrate whether pasteurization alters the milk sufficiently to prevent it from supplying the nutrients which we rely upon milk to furnish.

EXPERIMENTAL

The milk used throughout these experiments was received directly from the University of Wisconsin dairy each day. The source of milk was from approximately twenty farmers who sell their milk to the dairy. It was typical market type and contained about 3.7 per cent fat. One-half of the milk was pasteurized in the University dairy before bringing it to the laboratory. In the pasteurization process the milk was heated to 145° F. in a vat and held at that temperature for 30 minutes.* It was then cooled over a surface cooler to 40° F. The two milks were therefore identical except for the pasteurization process.

The first feeding trial, which will be described in detail, was started October 26, 1932. Three litters of six rats each were taken from the stock colony at weaning. The litters were divided as equally as possible according to weight and sex into two groups, one for the raw milk and one for the pasteurized. Each animal was put in an individual wire bottom cage and fed nothing but the milk supplemented with iron, copper, and manganese.

The iron was supplied as ferric chloride, the copper and manganese as the sulfates. The minerals were administered as follows:

First week: 0.5 mg. Fe + 0.05 mg. Cu per rat daily in milk.

Second week: 0.5 mg. Fe + 0.05 mg. Cu + 0.04 mg. Mn per rat daily in milk.

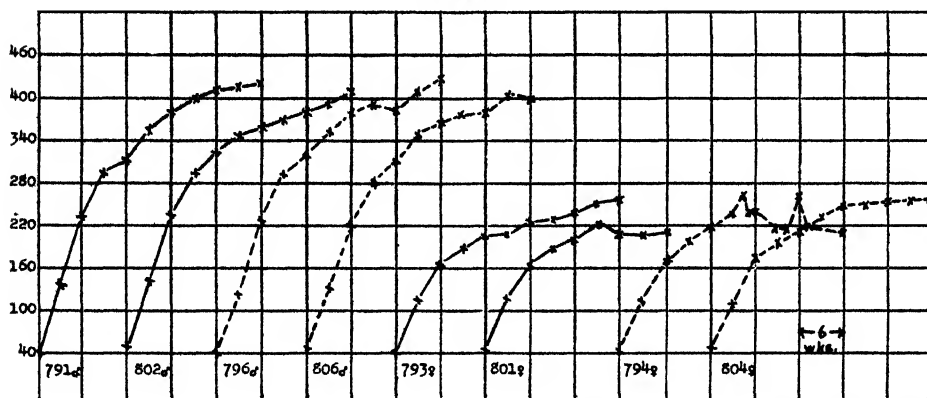
Third week: 1.5 mg Fe + 0.15 mg Cu + 0.15 mg. Mn per 100 cc. of milk.

The rate of mineralization outlined for the third week was continued throughout the duration of the experiment. The animals were fed *ad libitum* but during the first 2 weeks the minerals were placed in a small amount of milk fed in the morning and additional milk supplied in the evening. Beginning the 3rd week 35 cc. of mineralized milk were supplied in the morning and additional untreated milk in the evening depending upon the amount consumed the previous day. By this method consumption records were kept without leaving a great deal of unconsumed milk in the dishes over night. The animals were weighed weekly.

* Cherry-Burrell spray type pasteurizer used. Temperature manually controlled. Recording thermometer used.

All animals except one (Rat 805) grew exceedingly well on these diets and remained on the experiment for a period of 30 weeks. Rat 805 became sick during the fourth week and upon autopsy showed an enlarged and highly pigmented liver. Growth curves for two male and two female rats on mineralized raw milk and a like number on mineralized pasteurized milk are given in Chart I. The males grew from 60 to 200 gm. in about

CHART I.



Growth curves for rats grown on mineralized raw and pasteurized milk. The solid line indicates raw milk and the broken line pasteurized milk.

28 days, which is somewhat better growth than previously reported (3). It is readily seen from these curves that there is no difference in the growth of the rats on the pasteurized and raw milks. The fact that the rats on the pasteurized milk grew very well and were maintained for a period of 30 weeks demonstrates that there is no serious deficiency due to the heat treatment.

An idea of the physical condition of the animals may be gained from Fig. 1 showing the picture of Rat 791 on raw milk and Rat 796 on pasteurized milk. They had been on the milk diet nine weeks at the time the picture was taken and weighed within one gram of each other. They were in good flesh, their coats were smooth and sleek and their eyes bright.

When the animals had been on the experiment 10 weeks (14 weeks old) ovulation studies were made on the females by vaginal smears. All animals ovulated normally. Some of the animals were allowed to mate with production of young as may be seen from the growth record of Rat 794. Reproduction studies will not be included in this paper.

The only abnormal condition which we observed was the presence of some respiratory trouble during March and April. However, there was no indication that the animals on pasteurized milk were more susceptible, in fact Rats 792 and 801 both on raw milk had the most severe attacks. Although the temperature of our laboratory was kept very constant, the



FIG. 1. Photograph of two rats fed mineralized milk for nine weeks. Rat 791 (left) received raw milk reinforced with iron, copper, and manganese. Rat 796 (right) received pasteurized milk plus iron, copper, and manganese.

respiratory troubles might have been due to drafts. However, we were more inclined to believe that the difficulty was associated with some change in the winter supply of the milk. A second series of rats was started therefore on April 21, 1933. The experimental procedure was identical with that described for the first series. At this time of the year none of the animals grew quite as rapidly as those started in the fall and the animals receiving the raw milk made considerably better gains than those on pasteurized milk. This difference is represented in Table 1 which shows the

TABLE 1

Average daily gains in grams during the first six weeks of rats started in October and in April, 1932-1933

TIME OF STARTING EXPERIMENT	RAW		PASTEURIZED	
	Males	Females	Males	Females
October 26, 1932	4.55	3.04	4.36	3.04
April 21, 1933	4.00	2.66	3.49	1.91

average daily gain for the males and females during the first six weeks of each experiment.

Due to a very uneven distribution of sex in the litters used in the second experiment the number of animals in the male group receiving raw milk was reduced to one. In spite of this there seems to be no question but what the difference in growth between the fall and winter experiment is significant in all groups. This difference must be due to a seasonal varia-

tion in the milk. In order to test this suggestion more carefully three series of experiments were conducted during the past year.

The milk was obtained again from the University dairy. The average fat content varied from 3.67 per cent during the fall to 3.7 per cent in the spring. The rats were started on the following dates: October 14, 1933; December 27, 1933; and February 6, 1934. The average daily gains during the first six weeks for the different groups are given in Table 2.

TABLE 2
Average daily gains in grams during the first six weeks of rats started in October, December and February, 1933-1934

TIME OF STARTING EXPERIMENT	RAW		PASTEURIZED	
	Males	Females	Males	Females
October 14, 1933 . . .	4.19	2.88	3.90	2.59
December 27, 1933 . .	3.32	2.11	1.96	2.52
February 6, 1934 . . .	2.45	3.14	1.14	2.12

The rats started in October (1933) gave results quite similar to those obtained in October, 1932. Both the males and females grew a little slower and the differences between the raw and pasteurized milk were slightly greater than in the earlier series, but the differences cannot be considered significant. However, in the experiments started in December and February very significant differences are evident. In the case of the male rats there is a progressive decrease in the daily rate of growth from 4.19 to 2.45 gms. for the raw milk and from 3.90 to 1.14 gms. for the pasteurized milk. The females show some indication that the winter milk is of poorer quality than the summer milk. However, the results are not as uniform as for the males, and the females do not show the extremely poor growth in the February experiment that the males do. This we believe is due to the fact that the females do not grow as fast and therefore do not need as much of the factor or factors which winter milk is deficient in. Whatever the limiting factor is in winter milk, the deficiency is undoubtedly a relative one. Thus it is decreased sufficiently during winter feeding to have a very drastic effect upon the males which have a tendency to grow faster, but not enough to produce an equal growth impairment in females. The fact is further emphasized in the case of pasteurized milk. The milk produced in October and November, when the cows still have a store of nutrients from the summer feeding period, contains a fairly adequate supply of all needed nutrients. At this period, although pasteurization may destroy a small amount of certain constituents, the amount remaining is still sufficient to allow the animal an adequate supply. However, in December the supply of essential factors is reduced in the original raw milk and pas-

teurization will now increase the deficiency. Thus we have a decrease in the rate of growth of the males from 3.32 to 1.96 gm. per day due to the heat treatment. In February the raw milk allows a growth of only 2.45 gm. daily and pasteurization reduces the growth rate to 1.14 gm. It should be noted that the females on the pasteurized milk in both the second and third experiments grew faster than the males. This is further indication that the deficiency is more readily demonstrated in males than in females.

The female rats on raw milk during the February experiment also grew faster than the males but two of the rats in this group grew exceedingly well and raised the average. In this connection we might mention a test made on milk produced by two cows in the University herd and brought directly to the laboratory. This experiment was also started February 6 and the average growth for the males was 2.62 gm. which is almost identical with 2.45 found for the commercial milk at this time of the year. The average growth for the females was 2.65, which is lower than the 3.14 value but more in line with the other results for the females.

Season or the kind of feed ingested by the cow, therefore, has a much greater effect upon the nutritive value of milk than does pasteurization. Pasteurization will aggravate the deficiency of a poor milk, but a good milk, one produced when the cow is receiving an abundance of green feed containing certain essential factors, is little affected by pasteurization. Our problem therefore becomes one of producing a milk so complete that slight changes due to pasteurization have no effect on the nutritive quality.

DISCUSSION

The lack of any noticeable destructive action due to pasteurization of a good quality milk obtained in our studies with rats is in accord with the general conclusions reached in extensive studies with children in this country (1) and in Scotland (5). The Lanarkshire studies have been criticized, but a recent reexamination of the data by Elderton (6) substantiates the original conclusion that "There is no evidence that raw milk has an advantage over pasteurized or pasteurized over raw in increasing growth when the two are directly compared on this selected material."

The deficiency of winter produced milk and the increase in this deficiency due to pasteurization observed with rats indicates that there is a definite change in the composition of the milk during the winter period. Although it is well known that the amount of certain vitamins in milk varies with the season, it is difficult from our present results to determine what specific factors may be responsible for the gross changes observed. Vitamin C may be disregarded because rats do not require this factor preformed in the diet. Since a few of our animals suffered from respiratory troubles, it might be suggested that we were dealing with a vitamin A deficiency. However, there is no indication that our rats were receiving a

limited supply of this factor. In a recent paper Baumann, Steenbock, Beeson, and Rupel (7) calculated the vitamin A activity of Holstein milk produced in the University dairy herd to be approximately 21 International units per gram of butter fat when the cows were on winter feed. The rats showing growth impairment in our studies would therefore be ingesting at least 50–60 International units daily.

In order to demonstrate that the presence of the minerals had no destructive action on vitamin A, the carotene and vitamin A content of a sample milk was determined before mineralization and a similar sample after the milk had been mineralized and allowed to stand 24 hours. The values before mineralization were 7.5 gamma of carotene and 7.5 gamma vitamin A per gram of butterfat and after mineralization the value was 7.5 gamma carotene and 6.5 gamma vitamin A. Thus there was no inactivation due to the presence of the metals.

Krauss, Erb, and Washburn (4) found a destruction of about 25 per cent of the original vitamin B content of milk due to pasteurization. However there is no indication that either the B or G content of milk varies with seasonal change. Gunderson and Steenbock (8) concluded that the vitamin B content of milk was under physiological control and Dutcher, Guerrant and McKelvey (9) conclude in a recent paper that the vitamin B and G potency of raw milk is remarkably constant throughout the year. It is possible that the difference in winter and summer milk may be associated with change in the vitamin B₄ content but further work is necessary before any definite relationship can be established.

The studies which we have conducted so far suggest that the rate of growth of male rats on mineralized milk is an excellent measure of changes in the nutritive value of that milk. This method may be of considerable importance in measuring the value of milks produced by cows fed artificially dried hays and legumes preserved by the A. I. V. process during the winter months.

SUMMARY

1. Rats started on experiment in October and grown on mineralized raw milk and mineralized pasteurized milk showed no differences in growth or development over a period of 30 weeks.

2. The average daily gains during the first six weeks for rats on mineralized raw milk were less for the animals started in April than those started in October. In April the rate of growth for rats on pasteurized milk was inferior to that obtained on raw milk.

3. A decrease was observed in the daily rate of growth in male rats on mineralized milk from 4.19 gm. for milk produced in October to 3.32 gm. for milk produced in December to 2.45 gm. for milk produced in February. The decrease for male rats on pasteurized milk for the same periods was 3.90 to 1.96 and to 1.14 gm. The female rats showed some decrease in

growth on winter milk but the impairment in growth during this period was not nearly as great as that observed in the case of the male rats.

4. The kind of feed ingested by the cow has a greater effect upon the nutritive value of milk than does pasteurization.

5. Pasteurization has practically no detrimental effect, as measured with rats, upon the nutritive value of a milk of high nutritive quality but may further decrease the value of a milk of low nutritive quality.

BIBLIOGRAPHY

- (1) FRANK, L. C., CLARK, F. A., HASKELL, W. H., MILLER, M. M., MOSS, F. J., and THOMAS, R. C. Do children who drink raw milk think better than children who drink heated milk? U. S. Pub. Health Rep. 47, 1951. 1932.
- (2) STIRLING, J. D., and BLACKWOOD, J. H. The nutritive properties of milk in relation to pasteurization. Bull. No. 5, The Hannah Dairy Research Institute. 1933.
- (3) KEMMERER, A. R., ELVEHJEM, C. A., HART, E. B., and FARGO, J. M. The nutritive value and efficiency of mineralized milk. Amer. J. Physiol. 102: 319. 1932.
- (4) KRAUSS, W. E., ERB, J. H., and WASHBURN, R. G. The effect of pasteurization on some of the nutritive properties of milk. Ohio Agric. Exp. Sta. Bulletin, 518. 1933.
- (5) LEIGHTON, G., and MCKINLAY, P. L. Milk consumption and the growth of school children. His Majesty's Stationery Office, 1930.
- (6) ELDERTON, E. M. The Lanarkshire milk experiment. Ann. Eugenics 5: 326. 1933.
- (7) BAUMANN, C. A., STEENBOCK, H., BEESON, W. M., and RUPEL, I. W. Fat-soluble Vitamins XXXIX. The influence of breed and diet of cows on the carotene and vitamin A content of butter. J. Biol. Chem. 105: 167. 1934.
- (8) GUNDERSON, F. L., and STEENBOCK, H. Is the vitamin B content of milk under physiological control? J. Nutrition 5: 199. 1932.
- (9) DUTCHEE, R. A., GUERRANT, N. B., and MCKELVEY, J. G. Vitamin studies XX. The effect of various methods of pasteurization on the vitamin B and the vitamin G content of cow's milk. JOURNAL OF DAIRY SCIENCE 17: 455. 1934.

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THE IODINE CONTENT OF MILK AS AFFECTED BY FEEDING IODIZED DRY MILK*

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Interest brought about by the ever increasing usefulness of iodine in veterinary, as well as in human medicine, has resulted in considerable experimental work; however, conflicting reports on the value of feeding iodine to farm animals (1-5) have been numerous. Orr, Crichton and Middleton (1) fed calves three grams daily without harmful effects, but Forbes *et al.* (4) fed only ten milligrams per 100 pounds of body weight to calves with resulting rough coat and digestive disturbances. Malon, DuToit and Groenewald (3) believe that twenty milligrams per day for a year was responsible for the decreased birth rate and low vitality of lambs; whereas Veghelyi (5) has been able to show increased rate of growth and wool yield in sheep fed approximately one gram iodine daily. Orr and Leitch (6) indicate, as a possible cause of some of these discrepancies, that secondary conditions, character of diet in particular, may affect the metabolism of iodine very greatly. Those who have interested themselves in the question seem quite in agreement that iodine fed even to cows not suffering from iodine deficiency affects the milk yield favorably (7-10).

Iodine, like certain other constituents of the diet enters into the milk and seems to be transmitted according to the amount ingested (11-15). This fact seems to have suggested to several workers the possibility of producing a naturally combined organic form of iodine for human use, although what concentration of iodine may be attained in milk by feeding iodine to cattle, or what amount may be advisable does not find entire agreement among them. Wendt (16) states that it is difficult to increase the iodine content of milk beyond 100 parts per billion. However, others have been able to increase this value to a much higher level; McIlargue (17) reports 400 parts per billion after feeding 100 milligram doses and Forbes *et al.* (4) have found that feeding 1.2 grams per day yields milk and cream with a relatively high iodine content but with objectionable odor.

Most of the literature heretofore, has dealt with the concentration of iodine in the milk (2, 15, 17, 18, 19) or the partition of iodine among the milk constituents (20-26). Table 1 shows data presented by various in-

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investigators with recoveries as calculated by assuming average milk yield where actual milk yield was not indicated, or average food intake where that was not specified. Krauss and Monroe (27) state that "only 10 to 15% of the iodine appears in the milk." This seems to be a higher return than is shown by other published data.

TABLE 1
Iodine recovered in milk as indicated by published data

AUTHORITY	IODINE CONCENTRATION IN LIQUID MILK	IODINE BALANCE PER COW PER DAY		
		Iodine intake	Iodine secreted in milk	Recovered in milk
	(p.p.b.)	(mgs.)	(mgs.)	(per cent)
Erf (2)	333	499.0	2.780	0.550
McHargue (17)	400	76.5	3.200	4.100
Monroe (19)	100	765.0	1.178	0.154
Krauss & Monroe (27)	500	76.5	4.170	5.400
Corrie (15)	700	76.5	7.000	9.150
Scharrer & Schwaibold (23)	2120	459.0	21.200	4.620

Attempts to prove the relative efficiency of organic and inorganic iodides as a source of iodine in animal nutrition have not been entirely satisfactory (2, 27) and it appears that although the inorganic form may be made efficient by means of proper diet the organic form requires less consideration of secondary factors.

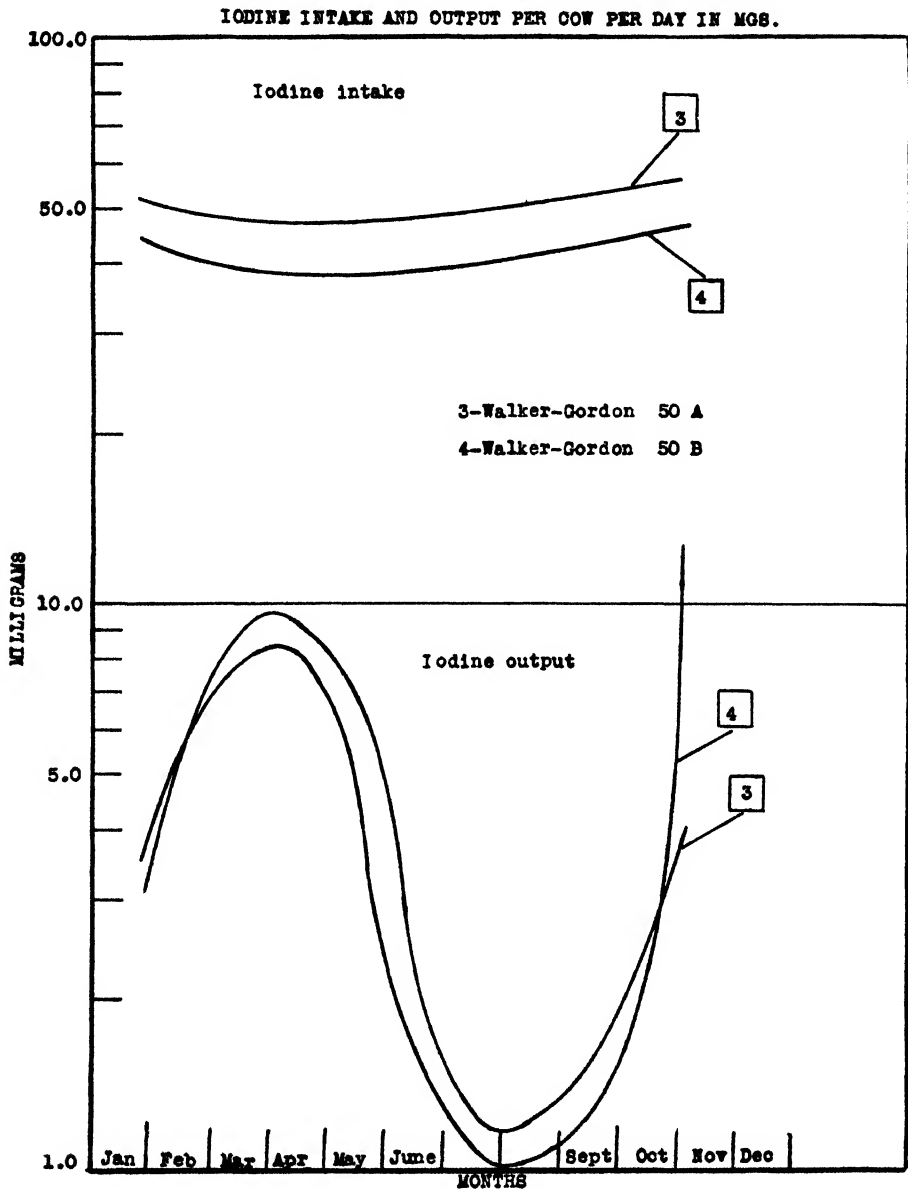
The primary object of the work summarized in this paper was to determine not only the iodine content of the milk of cows fed controlled and known amounts of an organic iodine but also the percentage of iodine recovered in the milk as affected by season and period of lactation.

EXPERIMENTAL

Experimental groups of cows at the Walker-Gordon farms were fed iodized dry milk containing predetermined amounts of iodine in conjunction with a normal stall ration consisting of roughage and concentrate. Iodine Suspensoid (Merek) was added to skimmed milk in such concentrations as to provide, when the milk was dried (28) and mixed in the concentrate, an empirical daily dosage of 50 milligrams or 250 milligrams of iodine per cow. This scheduled daily intake was not strictly adhered to however, as practical considerations in feeding management dictated slight departures in accordance with the milk produced by individual animals. Control groups were maintained on the normal stall ration free from added iodine. The cows under observation, including the controls, were not subjected to the fluctuations in feeding regimen to which the average producing herd is subject during transition from stall to pasture feeding and *vice versa*. Each of the groups were divided; one sub-group was composed of

animals in the early period of lactation and the other included only animals at a relatively advanced stage of lactation. Samples of the mixed

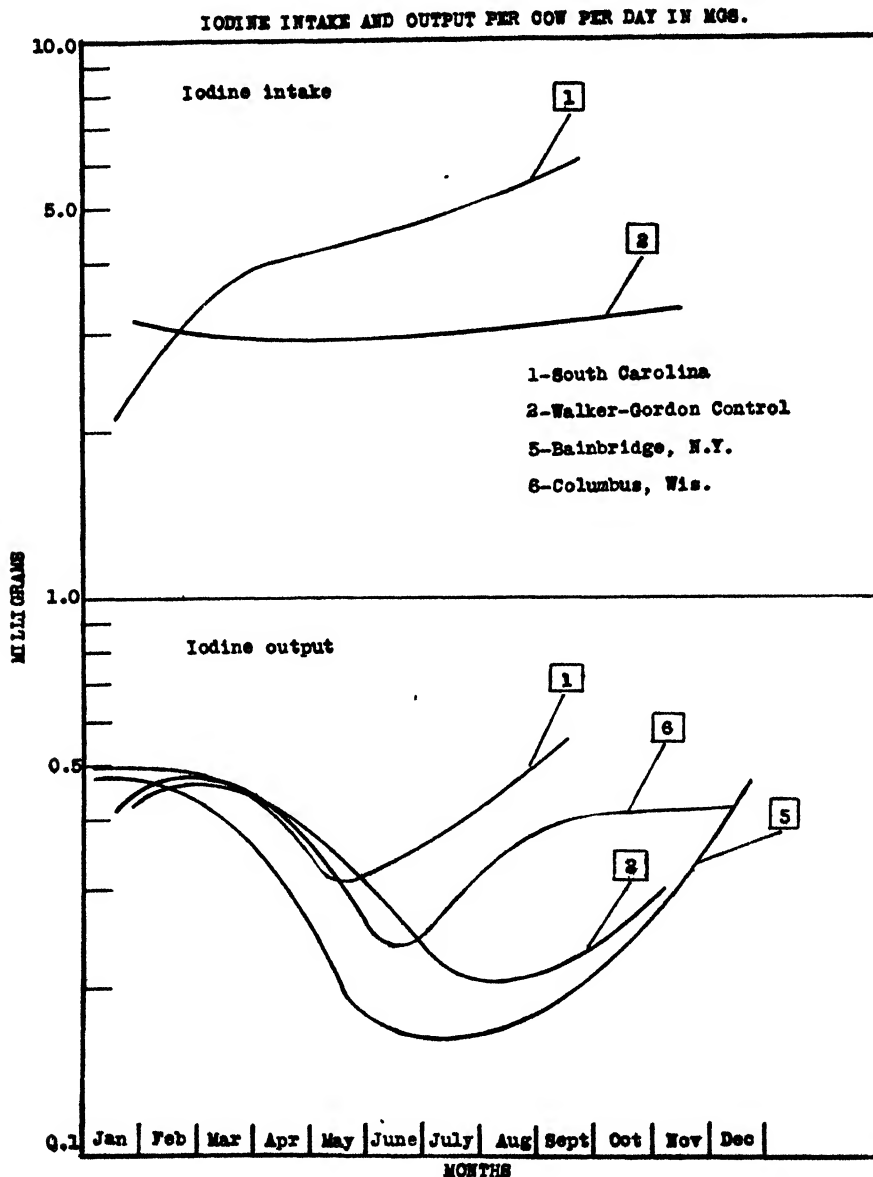
CHART I.



milk were taken for iodine determinations immediately before the experimental feeding was started and again after 10 days, 2 months, 3 months, 6 months, and 9 months. Cows receiving 250 milligrams per day were dis-

continued after the second month. No objectionable flavor or odor was reported in the milk. A modification of the Remington-McClendon method

CHART II.

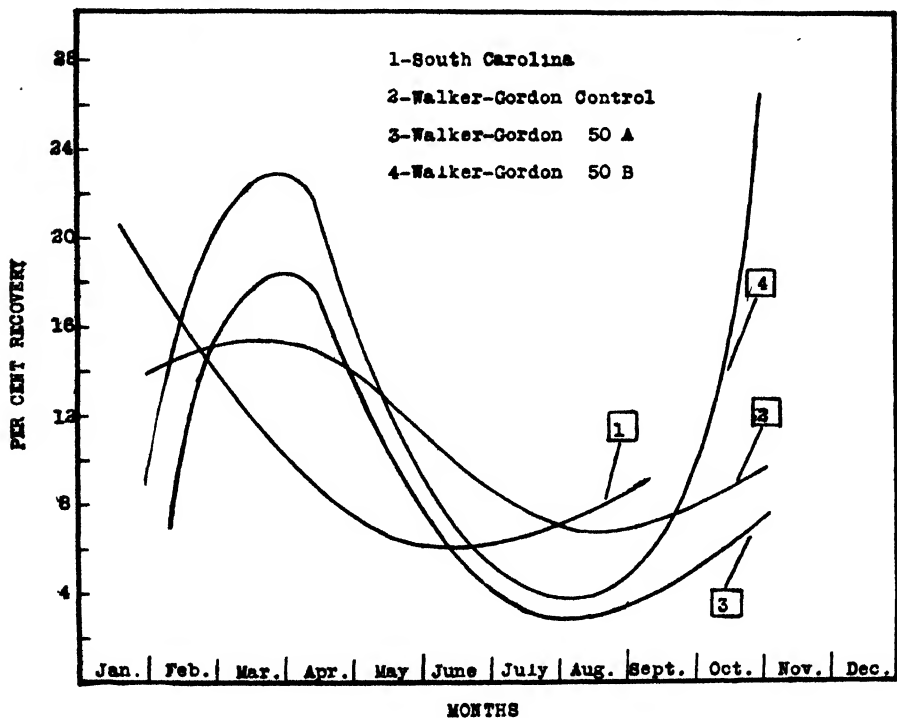


(29) was used for the iodine determinations. The summarized results from this controlled series of feeding experiments are shown in table 2. (Charts I, II and III).

There is some slight indication that non-pregnant cows early in lactation return a smaller proportion of ingested iodine in their milk than do those in an advanced stage of lactation. (Cows of less than 15 days' lactation have not been included because of the possible abnormal iodine content of the colostrum (30)). This relationship while not marked, nevertheless, seems to be consistent. Further work with groups more carefully selected as to stage of lactation and pregnancy must be done before definite quantitative conclusions may be reached.

CHART III.

PERCENTAGE IODINE RECOVERED IN MILK ACCORDING TO SEASON



The effect of season on the iodine content of milk, as well as on the percentage of iodine recovered in the milk is very distinct. Not only is the concentration of iodine in the summer milk of cows receiving the iodine supplement lower than that in the winter milk, but this relationship also holds true for the control groups not receiving the iodized milk supplement. Similar seasonal variations had been noted previously in the mixed milk of herds in the intensive producing areas of Wisconsin and New York (31). These earlier data obtained from the mixed milk of many herds has been summarized on the basis of the milligrams of iodine secreted in the milk per cow per day, using the average production per day per cow as the basis of

TABLE 2
Iodine recovered in milk from controlled feeding experiments

DATE	GROUP	AVERAGE MONTHS IN LACTATION	IODINE CONCENTRATION IN MILK (p.p.b.)	IODINE BALANCE PER COW PER DAY		
				Iodine intake (mgs.)	Iodine output (mgs.)	Recovered in milk (per cent)
1/31/33	Control		27	3.11	0.427	13.7
3/27/33	Control	9.7	37	2.96	0.457	15.4
7/31/33	Control	1.8	12	3.07	0.205	6.8
11/4/33	Control	5.7	22	3.22	0.289	9.0
2/13/33	50A	1.2	173	50.50	3.070	6.1
3/27/33	50A	3.2	585	51.78	9.600	18.5
5/5/33	50A	4.6	350	47.90	5.600	11.7
7/31/33	50A	2.6	82	48.00	1.160	2.38
11/4/33	50A	1.1	216	56.60	4.000	7.1
2/13/33	50B	12.1	290	43.40	3.490	8.1
3/27/33	50B	12.8	771	37.38	8.710	23.3
5/5/33	50B	9.0	498	43.47	7.150	16.4
7/31/33	50B	9.8	101	36.00	0.951	2.64
11/4/33	50B	10.1	961	48.10	12.760	26.5
2/13/33	250A	1.6	727	239.00	13.200	5.5
3/27/33	250A	2.7	2756	225.00	42.330	18.8
2/13/33	250B	13.1	1064	154.60	10.570	6.85
3/27/33	250B	15.2	2707	173.60	28.420	16.4

computation. The summary is shown in table 3 (Chart II). Change from pasture to stall feeding, as well as climatic conditions during the summer and winter months could have contributed to the results shown by these earlier data. The controlled feeding experiments at the Walker-Gordon Farms, however, preclude the effect of such change in feeding regimen as the direct cause of the relatively lower recovery during the warmer season.

TABLE 3
Daily iodine output per cow in the milk of Wisconsin and New York herds

WISCONSIN			NEW YORK		
Date	Iodine concentration in liquid milk	Daily iodine output per cow	Date	Iodine concentration in liquid milk	Daily iodine output per cow
	(p.p.b.)	(mgs.)		(p.p.b.)	(mgs.)
1/7/30	38.8	0.480	1/16/32	38.9	0.486
4/8/30	31.6	0.390	4/8/32	23.5	0.294
4/17/30	39.5	0.487	4/15/32	24.5	0.306
			4/22/32	33.5	0.419
6/16/30	17.1	0.212	6/10/32	13.1	0.164
9/9/30	33.8	0.418	9/10/31	15.7	0.196
			9/11/31	14.7	0.184
12/7/30	32.6	0.404	12/17/31	35.0	0.430
			12/21/31	39.2	0.490

Detailed analysis of the data obtained during a survey of the iodine content of milk produced in South Carolina (31) also shows a definite tendency toward a lower iodine content of the milk and a lower percentage recovery during the warmer months of the year. The seasonal fluctuation, however, is not as great as noted in the milk produced in the North. (Table 4; Charts II and III.)

TABLE 4
Average iodine recovered in milk produced in South Carolina (31)

MONTH	IODINE CONCENTRATION IN LIQUID MILK	IODINE BALANCE PER COW PER DAY		
		Iodine intake	Iodine recovered in milk	Iodine recovered in milk
	(p.p.b.)	(mgs.)	(mgs.)	(per cent)
January	61.3	2.14	0.41	20.67
March	73.8	3.80	0.48	14.50
May	47.2	4.27	0.29	6.26
September	94.3	5.88	0.56	9.00

SUMMARY

A critical inspection of the data presented herein shows substantial and consistent agreement in the trend of iodine recovery in the milk as affected

by seasonal or environmental conditions. The data have been collected at stated intervals over a period of three years in four different states. The records as shown in the tables and graphs represent numerous iodine determinations on the composited milk from thousands of cows, as well as numerous iodine determinations on composite feed samples and individual constituents of the ration.

The percentage of iodine recovered in the milk at comparable periods is essentially of the same magnitude, irrespective of whether the iodine ingested is in such a form as that naturally occurring in ration constituents produced on high iodine soils, as in South Carolina, that naturally occurring in normal ration constituents grown in other areas, or that supplied in the form of iodized dry milk.

The output of iodine in the milk per cow per day does not parallel the iodine ingested, irrespective of the intake level or form in which it is ingested, insofar as the data presented herein indicate. Nevertheless, the iodine concentration, as well as the total iodine output of the milk, is distinctly influenced and increased when an organic iodide supplement such as iodized dry milk is fed. It appears that the lack of a parallel relationship between the total iodine output and the percentage recovery in the milk on the one hand, and iodine ingested on the other, may be due primarily to environmental conditions prevailing during the relatively warm and cold seasons of the year. A distinctly lower total output and lower percentage recovery was found in all territories covered by these studies during the warm months.

REFERENCES

- (1) ORR, J. B., CRICHTON, A., AND MIDDLETON, W. A note on the toleration of farm animals for high doses of iodine. *Vet. Rec.* 9 [48]: 1055. 1929.
- (2) ERF, OSCAR. An eighteen year survey of the physiological effects of feeding iodine to high producing cows. Columbus, Ohio, Dec., 1930.
- (3) MALON, A. I., DU'TOIT, D. J., AND GROENWALD, J. W. Studies in mineral metabolism 20. Iodine in the nutrition of sheep. 18th Rep. Director Vet. Service and Animal Industry. Union S. Africa, p. 651, 1932, Onderstepoort, S. A.
- (4) FORBES, E. B., KARNS, GEO. M., BECHDEL, S. I., WILLIAMS, P. S., KEITH, T. B., CALLANBACH, E. W., AND MURPHY, R. R. The value of iodine for livestock in Central Pennsylvania. *J. Agr. Research* 45: 111. 1932.
- (5) VEGHELYI, ERNÖ. A jódetetés befolyása a szoptató anyajuhok és a bérányok fejlődésére, valamint a gyapjú hozamára és minőségére. *Mezogazdosági Kötetások* 4: 349. 1931. Cited by *Chem. Abst.* 26: 2492. 1932.
- (6) ORR, J. B., AND LEITCH, I. Iodine in practical agriculture. *Internat. Rev. Agric.* Pt. I. *Month. Bull. Agric. Science and Practice* 21 [6]: 197. 1930.
- (7) STINER. Bull. d. eidgenöss. Gesundheitsamtes 6: 23. 1934. Cited by Crichton, J. A., in *The mineral requirements of dairy cattle. J. Dairy Research* 2: 1. 1930.
- (8) NIKLAS, H., STROBEL, A., SCHARER, K. Zur Kenntnis des Jods als biogenes Element. II. Mitteilung: Fütterungsversuch mit steigenden Jodgeben zu Milchziegen. *Biochem. Z.* 170: 277. 1926.

- (9) SCHROPP, W. Zur Kenntnis des Jods als biogenes Element. XXI. Mitteilung: Fütterungsversuch mit Jod an Milchkühen. *Biochem. Z.* 213: 1. 1929.
- (10) STROBEL, A., SCHARRE, K., AND SCHROPP, W. Zur Kenntnis des Jods als biogenes Element. VII. Mitteilung: Fütterungsversuche mit verschiedenen Jodgaben an Milchkühen. *Biochem. Z.* 180: 313. 1927.
- (11) ROEMMLE, DR., AND MEYER, DR. Die Beeinflussung der Milchsekretion durch die Beifütterung von jodiertem Kochsalz unter Berücksichtigung der Allgäuer Verhältnisse. *Milchwirtschaft. Forsch.* 13: 59. 1932.
- (12) FEILENBERG, TH. VON. Joddüngung und Jodfütterung. I. *Biochem. Z.* 160: 210. 1925.
- (13) ORR, J. B., AND LEITCH, I. Iodine in nutrition. M. R. C. Special Rep. Series No. 123. 1929.
- (14) MIETHKE, M., AND SCHLAG, H. Jodstudie in Schleswig-Holstein. *Milchwirtschaft. Literaturber.* No. 52, p. 428. 1931.
- (15) CORRIE, F. E. Minerals in feeding of livestock. Notes on some recent iodine research. *Pharm. J.* 130: 361. 1933.
- (16) WENDT, GEORG VON. Jodierung der tierischen Nahrung [Helsingfors]. *Z. Volksernähr. Diätkost.* 8: 17. 1931.
- (17) MCHARGUE, J. S., ELY, F., ROY, W. R., AND YOUNG, D. W. The mineral content of milk from Jersey and Holstein cows during the period of lactation. 86th Meeting Am. Chem. Soc., Div. Biol. Chem. 1933.
- (18) CRICHTON, J. A. The mineral requirements of dairy cattle. *J. Dairy Research* 2: 1. 1930.
- (19) MONROE, C. F. The possibility of producing iodized milk. *Ohio Expt. Sta. Bull.* 13: 153. 1928.
- (20) MAGEE, H. F., AND GLENNIE, A. E. Studies on the effect of heat on milk. IV. The iodine content. *Biochem. J.* 22: 11. 1928.
- (21) MIETHKE, M., AND COURTH, H. Untersuchungen über den Jodgehalt der Kolostralmilch bei Kühen. *Milchwirtsch. Forsch.* 13: 394. 1932.
- (22) BLOM, I. J. B. Variations in the iodine content of grasses at different stages of growth and a note on the iodine content of milk. *Onderstepoort J. Vet. Sci. and Animal Ind.*, Vol. 2, No. 1, p. 139. 1934.
- (23) SCHARRE, K., AND SCHWAIBOLD, J. Zur Kenntnis des Jods als biogenes Element. XXIII. Mitteilung: Fortgesetzte Untersuchungen über den Chemismus des tierischen Jodstoffwechsels. *Biochem. Z.* 213: 32. 1929.
- (24) SCHARRE, K., AND SCHWAIBOLD, J. Cited by C. A. 24: 3541. 1930. The utilization of organic iodine compounds by cattle. *Tierernähr.* 1: 37. 1929.
- (25) SCHARRE, K., AND SCHWAIBOLD, J. Zur Kenntnis des Jods als biogenes Element. XVIII. Mitteilung: Über die Art der Bindung des Jods in der Milch. *Biochem. Z.* 207: 332. 1929.
- (26) ZICKGRAFF, G. Kann der Tierkörper Jodsalze assimilieren. *Z. Volksernähr. Diätkost* 6: 253. 1931.
- (27) KRAUSS, W. E., AND MONROE, C. F. A comparison of the influence of iodized milk and of potassium iodide administered directly on the size and iodine content of the thyroid gland of rats. *J. Biol. Chem.* LXXXIX, No. 2, p. 581. 1930.
- (28) HANFORD, Z. M., SUPPLEE, G. C., AND REMINGTON, ROE E. Studies on the iodine content of milk. I. Effect of desiccation and storage. *JOUR. DAIRY SCIENCE*, Vol. XVII, No. 1, p. 15. 1934.
- (29) REMINGTON, ROE E., MCCLENDON, J. F., AND VON KOLNITZ, HARRY. The determination of traces of iodine. V. Further refinements in technique. *J. Am. Chem. Soc.* 53: 1245. 1931.

- (30) BUNGER, DR. Verhandlungen der 52 (ordent). Hauptversammlung des Verbandes Deutscher Landwirtschaftlicher Versuchsstationen in Wiesbaden am 24 September 1932: Bericht des Ausschusses für Milchwirtschaft. Landw. Vers. Sta. 115: 63. 1933.
- (31) REMINGTON, ROE, E., AND SUPPLEE, G. C. Studies on the iodine content of milk. II. Variations in the mixed milk of herds. JOUR. DAIRY SCIENCE, Vol. XVII, No. 1, p. 19. 1934.

CONTROLLING PHYSICAL PROPERTIES OF HIGH SOLIDS MIXES*

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The sale of ice cream having a relatively high total solids content is increasing rapidly in the New England States. For the past several years the popularity of ice creams with 18 and 20 per cent butterfat has grown steadily. At the present time these rich ice creams are a considerable part of the total volume of ice cream made in the New England sector.

This high solids ice cream was first sold almost entirely by small ice cream companies attempting to market a high quality product. These companies retailed the bulk of their product through attractive stores or dairy cottages located in densely populated areas or along heavily traveled highways. Public demand was created for this type of ice cream and now practically all ice cream companies make the product. Lower ingredient costs probably have stimulated the manufacture of rich ice creams. Public preference for the product is largely due to its rich, creamy flavor and the entire absence of a "serum solids" taste since powdered or condensed milk products are used sparingly or not at all.

Variations in fat content from 16 to 25 per cent, with occasional ice creams running even higher, have been observed in the past few years. At the present time, however, the industry seems to be standardizing on 18 and 20 per cent butterfat, more often 20 per cent, for high fat ice creams. For this reason the work reported here has been confined to ice creams of 18 and 20 per cent butterfat.

In a previous publication¹ of the Massachusetts State College several problems arising in the manufacture of high butterfat ice creams were discussed and solutions were suggested. However, three serious problems remained unanswered; one was an excessive viscosity which interferes with efficient homogenization, cooling, freezing, and packaging; the second was a very unsatisfactory melting appearance; and the third, a crumbly body which makes serving of the ice cream difficult. All these difficulties became even more serious when butter, frozen cream, or plastic cream were substituted for sweet cream. In fact, many manufacturers use only sweet cream and whole milk as sources of fat in order to minimize the difficulties just enumerated.

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** The author wishes to express his appreciation to Dr. William H. Davis for his kindness in preparing the photomicrographs.

¹ J. H. Brockschmidt, 1933. The Manufacture of High Butterfat Ice Cream. Master's thesis, Mass. State College.

In this study an attempt has been made to solve the previously mentioned problems to the extent that any desired combination of butterfat sources might be used in high solids mixes without impairment of the finished product. Commonly accepted procedures were used throughout the work in processing, freezing, hardening, judging, and in making the necessary laboratory determinations. Any deviation from accepted procedures will be mentioned in the discussion of experimental results.

EXPERIMENTAL RESULTS

When high solids mixes are made, following the usual processing methods, so great a viscosity results after homogenization that the mix thickens on the surface cooler to the extent that a temperature of 50° F. or lower cannot be secured. Consequently many manufacturers decrease the homogenization pressure to 800 to 1000 pounds for a 20 per cent fat mix. At this low pressure the fat is not emulsified sufficiently to secure a smooth texture and prevent churning of the fat when freezing is too slow. In preliminary trials it was found that high pasteurization and homogenization temperatures (160 to 165° F.) decreased the viscosity only to a limited extent. The practice of two-stage homogenization, which largely destroys clumping in mixes of average composition, has only a slight effect in decreasing viscosity in high fat mixes. Microscopic examinations revealed that clumping after two-stage homogenization was sufficiently extensive to cause the extremely high viscosity of these mixes. In the study previously cited, data were given to show that butter, frozen cream, and plastic cream could be substituted for sweet cream by first making a "reconstructed cream" (emulsifying the butter, etc., in skim milk or whole milk by homogenization at a pressure of 1000 pounds and a temperature of 145° F.) which is used in the mix in the same way that sweet cream is utilized. This method has the disadvantage of being time consuming and laborious. Furthermore, it does not solve the problem of excessive viscosity which often is troublesome when sweet cream is used as the source of fat. In this experiment viscosity has been controlled successfully by passing the mix through three homogenizing valves (Fig. 1).

An examination of tables 1 and 2 reveals that reasonably high homogenization pressures can be used with high fat mixes even when butter is the chief source of fat if three-stage homogenization is practiced. Mixes 1 and 2, (one- and two-stage pressures), in either series were so viscous that they could not be cooled satisfactorily and the high viscosity interfered with every subsequent operation. However, mix No. 3, three pressures, had the viscosity of a product of average composition. The use of the first and third valves (Mix No. 4) decreased viscosity considerably but microscopic examinations revealed that the fat was improperly emulsified. The presence of many extremely large globules probably accounts for the poorer

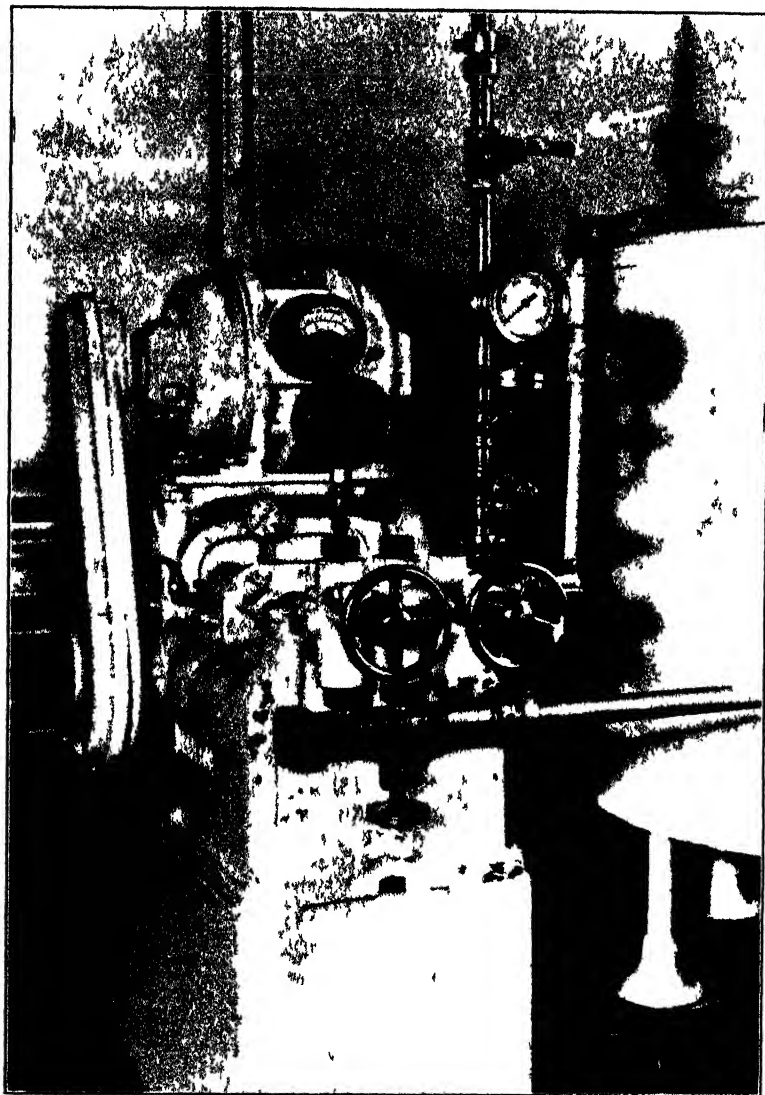


FIG. 1. The homogenizer used in this experiment was a two stage machine made by the Manton-Gaulin Manufacturing Company. The third stage, as is shown in the illustration, consisted of a pressure reducing valve (known as the Snow Viscosity Regulator). This valve was placed in the discharge line from the homogenizer.

quality of Mix No. 4 when compared with No. 3. A few of these large globules are visible in the accompanying photomicrograph. The great difference in viscosity between mixes 2 and 3 (expressed in $^{\circ}\text{M}$ at 68°F . with the McMichael Viscosimeter, No. 30 wire) clearly indicates the effectiveness of the third valve in decreasing viscosity.

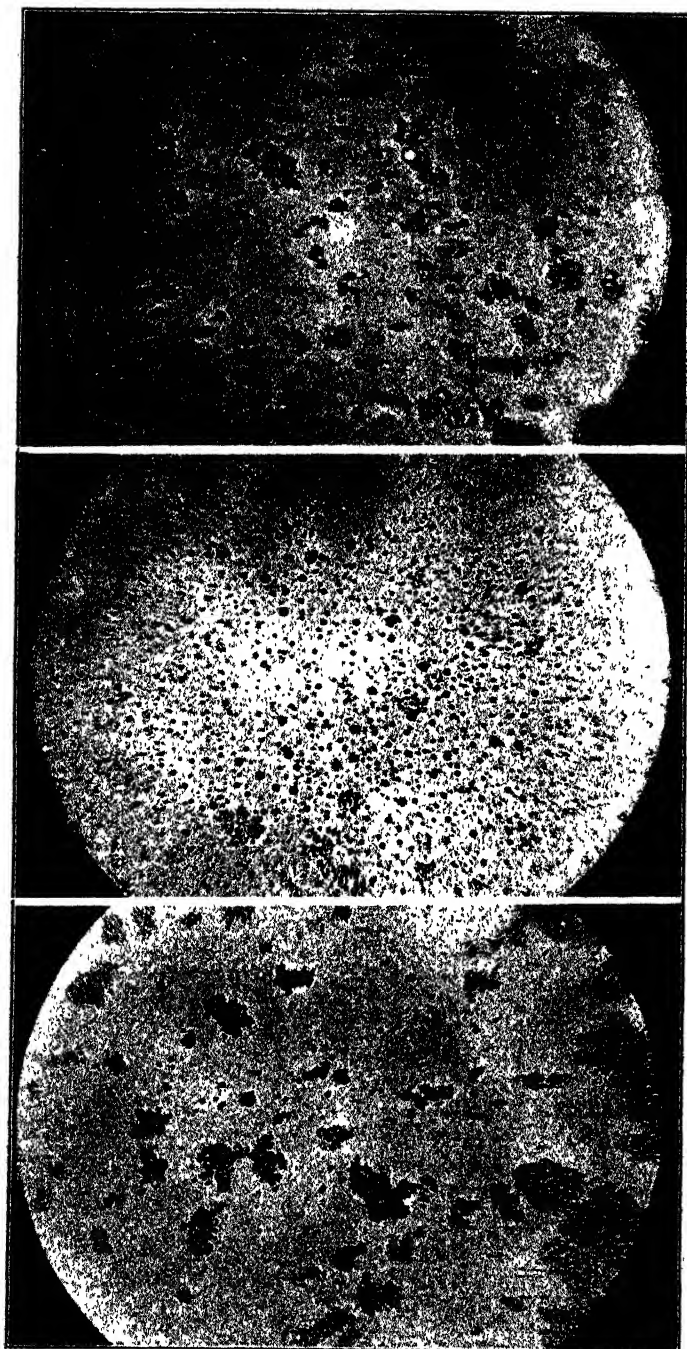


Fig. 2. Photomicrographs of diluted ice cream mix testing 18 per cent fat. The mixes illustrated are the same as Nos. 2, 3, & 4 in Table 1. The homogenization pressures were:

1. 2000 lbs. 1st stage and 500 lbs. 2nd stage.
2. 2000 lbs. 1st stage, 500 lbs. 2nd stage and 150 lbs. 3rd stage.
3. 2000 lbs. 1st stage and 150 lbs. 3rd stage.

Reduction of viscosity by three-stage homogenization makes it possible to cool the mix 5 or 6° F. colder. This in itself means less viscosity increase during aging.² The lower viscosity and greater dispersion of the butterfat undoubtedly account for the slightly faster freezing of the mixes homogenized through three valves. Faster freezing and more thorough distribution of fat probably are the causal factors in improving the texture and melting appearance of the ice cream subjected to three-stage homogenization.

Freezing trials have been checked on a direct expansion freezer which finishes a batch in about 25 per cent less time than the brine freezer used regularly in this experiment. With the more efficient freezer very little freezing time was saved by three-stage homogenization of the mix. However, the ice cream could be drawn from the freezer at a slightly lower temperature, which in itself is conducive to superior texture.

Microscopic examination of dilute solutions of mix in distilled water (1-100 dilution) revealed that three-stage homogenization produced an emulsion of uniformly small fat globules with clumping almost entirely absent. The few clumps which were present were extremely small, consisting of only a few fat globules to a clump. The photomicrographs fully substantiate this statement. (See Figure 2). Mixes 1 and 2 were so much alike under the microscope that a photograph of No. 2 only is included. The picture of No. 4 (2000 lbs. 1st stage and 150 lbs. 3rd stage) shows the presence of some very large globules, indicating that this method of homogenization did not thoroughly disperse the butterfat.

The melting appearance of ice cream high in butterfat is almost certain to be undesirable unless precautions are taken to avoid this difficulty. The ice cream often does not melt, appears "curdled" or "feathered," and "wheys off." The appearance is more suggestive of a whipped cream than ice cream. The unusually high ratio of fat to serum solids undoubtedly decreases the stability of the proteins, causing the feathery appearance and the whey separation, while the high percentage of fat tends to make the product rigid. However, the fat to serum solids ratio cannot be corrected by increasing the serum solids because the total solids content is already high and the increase in lactose concentration would hasten the occurrence of "sandiness." Therefore correction of the melting appearance must be accomplished in some other way. The homogenization treatment previously discussed (three successive pressures) improves the melting characteristics somewhat. Increasing the sugar content by one to two per cent entirely corrects the melting appearance. The lower freezing point and higher soluble solids content thus secured bring about normal melting.

² W. S. Mueller, 1933. Aging Effects on Gelatin Dispersions. Jour. Ind. & Eng. Chem., Vol. 25, No. 6, p. 707.

A slight increase in sweetness, in conjunction with the high fat content, seems desirable from the flavor standpoint. The author knows of several instances where the cane sugar content has been increased to 16 and 16.5 per cent because of consumer preference for the sweeter ice cream. Excessive sweetness can be prevented, however, by substituting corn sugar (cerelese) for a part of the cane sugar. The sugar content may be adjusted as in the mixes listed in tables 1 and 2, where to a base of 13 per cent cane sugar, 3 and 3.5 per cent, respectively, of corn sugar were added. The sweetness in terms of cane sugar in these mixes is between 15 and 15.5 per cent. Various sugar concentrations were used in this experiment and

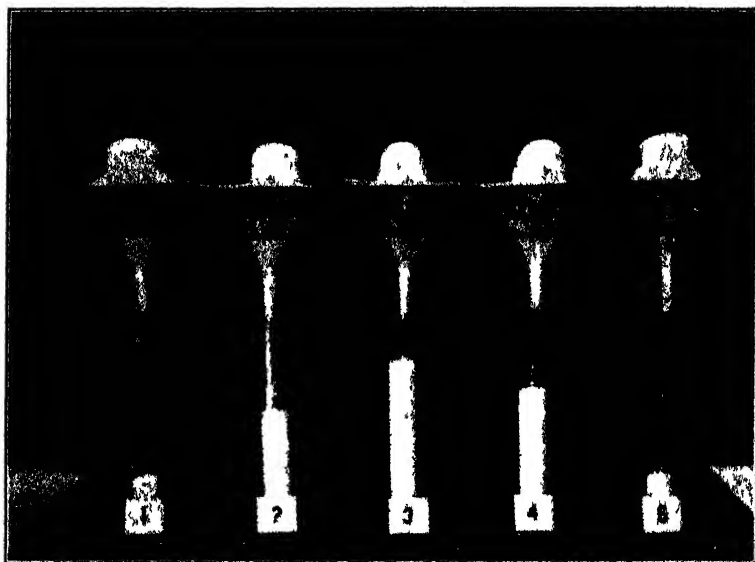


FIG. 3. The melting appearance of 20 per cent fat ice creams. Samples 1, 2, 3, & 4 are the same as those given in Table 2. The composition of the mixes is given in Table 2. The homogenization pressures follow:

1. 1500 lbs. 1st stage.
2. 1500 " " " plus 500 lbs. 2nd stage.
3. 1500 " " " " " " " " and 150 lbs. 3rd stage.
4. 1500 " " " " 150 " 3rd "
5. Same as 3 but contains 15 per cent cane sugar.

it was evident that the sugar content must be adjusted according to the fat content if a desirable melting appearance is to be secured. However, 16 to 17 per cent sugar proved sufficient.

The third defect previously mentioned, a crumbly body, which so often occurs in high butterfat ice cream, can be corrected in the same way that the melting appearance is made satisfactory. The corrective is increasing the sugar content. Increasing the serum solids content would probably prevent crumbliness, but this cannot be done for the reason previously

TABLE 1

*Different homogenization treatments with an 18 per cent butterfat mix**

MIX NO.	1	2	3	4
Homogenization pressure				
Lbs. on 1st stage	2000	2000	2000	2000
“ “ 2nd “		500	500	
“ “ 3rd “			150	150
Mix temp. leaving cooler	54° F.	54° F.	47° F.	48° F.
Relative viscosity (aged 24 hrs.)	560	575	55	74
Fat globules in clumps (estimated)	90%	75%	5-10%	50%
Min. to reach 100% Overrun	8	8	6.5	7
Body	Crumbly	Crumbly	Good	Good
Texture score (25 perfect)	22	23.5	24.5	23
Melting appearance	Curdy Melts slowly	Curdy Melts slowly	Smooth Creamy	Slightly curdy

* The composition of the mix was 18 per cent fat, 6.5 per cent serum solids, 16 per cent sugar, and 0.3 per cent gelatin. The ingredients of the mix were butter (75 per cent of the fat was obtained from butter), sweet cream, skim milk, powdered skim milk, 13 per cent cane sugar, 3 per cent corn sugar, and gelatin.

TABLE 2

*Different homogenization treatments with a 20 per cent butterfat mix**

MIX NO.	1	2	3	4
Homogenization pressure				
Lbs. on 1st stage	1500	1500	1500	1500
“ “ 2nd “		500	500	
“ “ 3rd “			150	150
Mix temp. leaving cooler	56° F.	56° F.	50° F.	50° F.
Relative viscosity (aged 24 hrs.)	580	564	130	148
Fat globules in clumps (estimated)	95%	95%	20%	65%
Min. to reach 100% Overrun	10	9.5	8.0	8.9
Body	Crumbly	Crumbly	Good	Fair
Texture score (25 perfect)	24	24.25	25	24
Melting appearance	Melts slowly Wheys off	Melts slowly Wheys off	Creamy Smooth	Curdy

* The composition of the mix was 20 per cent fat, 5.6 per cent serum solids, 16.5 per cent sugar, and 0.3 per cent gelatin. The ingredients of the mix were butter (two-thirds of fat), sweet cream, skim milk, 13 per cent cane sugar, 3.5 per cent corn sugar, and gelatin.

stated. Increasing the gelatin content also would prevent crumbliness. However, a high gelatin content is undesirable because it would prevent melting and increase the melting resistance in the mouth. This would be particularly undesirable in an ice cream of so high a total solids content.

A sugar content of 16 to 17 per cent is sufficient to prevent a crumbly body. Another advantage of the higher sugar concentration is that the ice cream is not excessively hard at the usual dipping and serving temperatures. If the rich, creamy flavor of high butterfat ice cream is to be fully

appreciated, the product must be reasonably soft when served. Preliminary experiments indicate that the ice cream should not be colder than 18° F. when served.

Difficulty is often experienced with excessively high viscosity when chocolate ice cream mixes are prepared. Therefore, several chocolate mixes were subjected to three-stage homogenization with virtually the same results as those already reported with high butterfat mixes. The composition of the chocolate mixes was 14 per cent total fat, 9 per cent serum solids, 17 per cent sugar, 0.3 per cent gelatin, 3 per cent Dutch process cocoa, and 43.4 per cent total solids. Three-stage homogenization greatly decreased the viscosity when compared with two-stage homogenization, made more efficient cooling possible, largely destroyed fat globule clumping, and improved the melting appearance. The rate of whipping was not changed to any appreciable extent but it was possible to cool the chocolate mixes (which were treated with three pressures) more rapidly in the freezer and draw them from the freezer at a slightly colder temperature than the control mixes.

A few trials have been run with mixes of average composition, subjecting them to three-stage homogenization pressures. An insufficient amount of data has been accumulated and no conclusions can be made. The differences, if any, with the lower fat mixes are evidently not marked.

As is shown in Figure 1, the third homogenizing valve consisted of an attachment known as the "Snow Viscosity Regulator." Presumably any type of reducing valve of appropriate size which is manually adjustable would do the same work. The writer has been informed that similar results are being secured in a commercial plant by soldering into the sanitary pipe carrying the mix from the homogenizer to the cooler a metal disc with a small hole in it. Evidently enough pressure builds up between the homogenizer and the metal disc so that the mix is forced through the hole with enough force to destroy fat globule clumping in the mix.

SUMMARY

1. High solids ice cream mixes, when made under usual processing conditions, are excessively viscous and produce crumbly ice cream possessing an undesirable melting appearance.

2. The use of butter, frozen cream, or plastic cream in place of all or a part of the sweet cream needed to supply the butterfat markedly increases these defects.

3. The use of three successive stages of homogenization entirely eliminates the problem of excessive viscosity and decreases the other defects already named. Pressures of 2000, 500, and 150 pounds are suggested as satisfactory maximum pressures for the first, second, and third valves, respectively, when homogenizing an 18 per cent butterfat mix. With a

20 per cent fat content, somewhat lower pressures of 1500, 500, and 150 pounds are suggested as maximum pressures.

4. A crumbly body may be prevented in high butterfat ice creams by increasing the sugar content to 16 to 17 per cent, depending somewhat upon the fat content of the mix. If the use of cane sugar alone produces an excessively sweet taste, the substitution of corn sugar for 3 to 4 per cent of the cane sugar is recommended.

5. Increasing the sugar content to 16 to 17 per cent improves the melting appearance of high fat ice creams and reduces the melting resistance of such ice creams.

6. Three-stage homogenization entirely eliminates the excessively high viscosity which invariably occurs in chocolate ice cream mixes of high solids content.

A STUDY OF SOME FACTORS INFLUENCING THE HILL CURD TEST*

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INTRODUCTION

Fairly extensive use is being made at the present time of the Hill test as a means of determining the curd tension of milk. For the most part the technique as recommended by Hill (1) has been followed closely. Several modifications in the procedure, however, have been suggested to date. Monier and Sommer (2) have recommended a change in the method of adding the coagulant, while Morris and Richardson (3) suggested running the test at a higher temperature.

A variation in the concentration of the calcium chloride solution also has been noted in written directions for the test. For comparative purposes it is important to know what influence slight variations in the technique will have on the final results. A question frequently asked is how closely will the results of different operators agree when working under similar conditions.

The present investigation was planned to determine the influence of certain modifications in technique on the results of the test. No evidence is available in the literature to show the normal limits of variation for the Hill test.

PLAN OF EXPERIMENT

To study the effect of certain modifications in technique, a relatively large number of parallel tests were run on the same sample of milk. Considerable care was taken to keep all factors constant except the one under observation.

A water bath accommodating thirty-six coagulation cylinders was used throughout. In this way it was possible to make thirty-six determinations at one time under identical conditions with respect to temperature. The temperature in all cases was 35° C. except where the influence of this factor was being studied. The milk, coagulation cylinders, and coagulant were all brought to the standard temperature prior to making the test.

The coagulant was freshly prepared for each series of thirty-six determinations. The 1-3000 granular pepsin was weighed out and added to sufficient distilled water to make a 0.6 per cent solution, except when the strength of the coagulant was varied. To insure that the pepsin was in solution, it was allowed to dissolve in the water for a period of ten minutes prior to use.

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TABLE 1
The effect of the individual operator on the results of the Hill curd test using four different lots of milk

EXPERIMENT NO	1			2			3			4		
	A	B	C	A	B	C	A	B	C	A	B	C
No. of Determinations	36	36	36	36	36	36	36	36	36	36	36	36
Curd Tension in grams												
Maximum	25.0	26.0	27.0	62.0	58.0	61.0	83.0	76.0	79.0	138.0	130.0	125.0
Minimum	14.0	17.0	15.0	32.0	30.0	31.0	50.0	52.0	50.0	100.0	94.0	99.0
Average	20.3	22.9	22.0	46.1	45.1	38.8	64.0	64.5	61.7	119.0	113.8	112.6
Coefficient of Variability												
Per Cent	13.3	13.7	14.0	17.7	14.8	17.7	12.3	17.7	12.2	8.9	7.5	8.6
Maximum Average Difference Between Operators Divided by the Probable Error of Difference		1.75			6.1			2.5			4.3	

The calcium chloride solution, except when it was the variable, was prepared according to Hill's directions (1) and was added to the pepsin solution just prior to use. After thorough mixing, 10 cc. of the coagulant was pipetted into the coagulation cylinders and 100 cc. of milk added. This modified technique, first suggested by Monier and Sommer (2), was found to be as accurate and is more rapid than the procedure recommended by Hill.

The curd-o-meter, which cuts down through the curd and permits the making of a large number of determinations in a comparatively short time was used throughout the study.

The time interval between the addition of the coagulant and cutting the curd was checked with a stop watch. The curd tension of all samples was determined at the end of ten minutes unless otherwise stated.

RESULTS

Personal Factor

In tables 1 and 2 are recorded the results obtained with three different operators testing four different lots of milk. In each series of thirty-six determinations, twelve were made by each operator, although no operator was allowed to determine the curd tension of more than six samples in succession. Each operator was thoroughly familiar with the test and thus

TABLE 2

Showing the variations in 432 curd determinations made by three different individuals

OPERATOR	A	B	C	MAXIMUM AVERAGE DIFFERENCE BETWEEN OPERATORS DIVIDED BY THE PROBABLE ERROR OF DIFFERENCE
No. of Determinations	144	144	144	
Average Curd Tension in Grams	62.34	61.59	58.78	1.24

any variations in the results between the different operators can be ascribed to differences due to the personal factor.

An inspection of the data presented in table 1 shows rather wide variations between the extremes for each lot of milk. These variations were not confined to any one operator but are evident in all three. The coefficient of variability expressed in table 1 gives some indication of the variation to be expected in running the test, irrespective of the operator. In a practical test of this kind such variations are to be expected and are beyond the control of the operator.

In table 1, and most of the succeeding tables, the actual difference in the average results of two series of curd determinations has been divided by the probable error of the difference; if the quotient exceeds 4.0 it is fairly

safe to conclude that the variable being studied has introduced a measurable variation in the results. It will be noted that in experiments 2 and 4, table 1, the maximum differences in results between the three operators are large enough to be statistically significant. This would indicate that the individual operator may be a factor influencing the results of the test but is not necessarily so.

In Table II all of the results have been considered collectively. The average results for all samples agree very closely and indicate that if the test is performed in careful manner the variation between individual operators need not be significant.

TEMPERATURE

Two experiments were run in which the temperature of the test was varied, other factors remaining constant. The results are recorded in table 3. The results of both experiments point to the fact that tempera-

TABLE 3
The effect of temperature on the results of the Ull curd test

EXPERIMENT	TEST NUMBER	TEMPERATURE USED DEGREES C.	AVERAGE CURD TENSION IN GRAMS	DIFFERENCE* DIVIDED BY THE PROBABLE ERROR OF THE DIFFERENCE
A	36	30.0	39.1 \pm .38	- 8.40
	36	35.0	57.6 \pm .55	
	36	37.5	67.8 \pm .60	+ 4.00
	36	40.0	70.3 \pm .64	+ 4.70
B	36	35.0	38.72 \pm .39	
	36	37.5	46.57 \pm .42	+ 4.33

* Average curd tension at 35° C. was used as a standard.

ture is an important factor influencing the results of the curd test. A deviation in temperature of 2.5° C. above the standard temperature resulted in a marked increase in the average curd tension. These data leave no doubt as to the necessity of controlling the temperature factor very closely.

It was observed also that when the test was performed at a temperature in excess of 35° C. there was a marked tendency for the samples to whey off and pull away from the sides of the jar. This condition was objectionable and made uniform cutting of the curd exceedingly difficult.

TIME INTERVAL BETWEEN ADDING THE COAGULANT AND CUTTING THE CURD

In table 4 are recorded the results of two experiments performed with two lots of milk in which the time interval was varied between adding the coagulant and cutting the curd.

The results of both experiments show that a deviation in the time interval has an influence on the curd tension. The effect of the time factor

appears to be greater, however, with milk showing an average curd tension of 55–60 grams than with milk of a lower curd tension. It is evident that the time interval should be carefully controlled in making this test.

INFLUENCE OF THE AMOUNT AND CONCENTRATION OF COAGULANT

In table 5 are recorded the results of three experiments with three different lots of milk in which either the amount or concentration of coagulant was varied. In Experiment A the concentration of the coagulant solution was varied, a 0.3 per cent pepsin solution being compared with a 0.6 per cent solution, while the regular amount of a standard calcium chloride solution was used. The differences in curd tension, while not statistically significant, are of interest in that the milk coagulated with the 0.3 per cent pepsin solution showed a higher average curd tension than the samples to which the standard 0.6 per cent pepsin solution was added.

TABLE 4

The influence of variations between the time of adding the coagulant and cutting the curd on the results of the Hill curd test

EXPERIMENT	TESTS NUMBER	TIME INTERVAL IN MINUTES	AVERAGE CURD TENSION IN GRAMS	DIFFERENCE* DIVIDED BY THE PROBABLE ERROR OF THE DIFFERENCE
A	36	8	34.80 \pm .35	- 2.45
	36	10	38.78 \pm .39	
	36	13	41.32 \pm .27	+ 1.70
B	36	8	49.61 \pm .35	- 4.14
	36	10	56.94 \pm .44	
	36	13	63.38 \pm .45	+ 3.25

* Average curd tension at the end of 10 minutes used as a standard.

In Experiment B the standard coagulant solution was used throughout but the amount of coagulant added was varied from 5 to 15 cc. per sample. To eliminate the dilution effect all were made up to the same volume with distilled water. The results show the same trend as was shown in the other experiment, viz., that a decrease in the amount of coagulant resulted in an increase in the curd tension. Reducing the amount of coagulant solution by 50 per cent of the standard amount increased the average curd tension of the samples tested 15.67 grams. This difference is statistically significant. An increase in the amount of coagulant over the standard amount resulted in a decrease in the average curd tension amounting to 5.42 grams. This observation is in harmony with the results of Lind and Jensen (4). These workers studying the effect of rennet on the contraction of the curd state, "if the addition of rennet is increased beyond a certain limit the firmness and coherence of the curd do not grow but on the contrary diminish."

In Experiment C the regular amount of pepsin solution was used but the amount of calcium chloride solution was varied. The entire elimination of the calcium chloride produced a marked reduction in the curd tension. When one-half the regular amount of calcium chloride was used the curd tension was increased. An increase in the amount of calcium chloride solution beyond the standard amount did not materially influence the curd tension. These data indicate that the amounts of calcium chloride or pepsin used may influence the curd test, particularly if the amount of coagulant is reduced.

TABLE 5
The effect of the amount of coagulant used on the results of the Hill curd test

EXPERIMENT	TESTS NUMBER	AMOUNT OF COAGULANT USED	AVERAGE CURD TENSION IN GRAMS	DIFFERENCE DIVIDED BY THE PROBABLE ERROR OF DIFFERENCE
A	72	10 cc. 0.3% Pepsin Solution	48.43 \pm .44	+ 2.02
	72	10 cc. 0.6% Pepsin Solution	44.11 \pm .51	
B	36	5 cc. Standard Coagulant Solution Plus 10 cc. Water	67.25 \pm .49	+ 6.8
	36	10 cc. Standard Coagulant Solution Plus 5 cc. Water	51.58 \pm .54	
	36	15 cc. Standard Coagulant Solution	46.16 \pm .45	- 2.4
C	36	No Calcium Chloride Used	29.4 \pm .81	- 6.97
	36	One-half Regular Amount of Calcium Chloride Used	62.2 \pm .70	+ 5.00
	36	Regular Amount of Calcium Chloride Used	49.5 \pm .42	
	36	Double the Regular Amount of Calcium Chloride Used	30.6 \pm .44	0.0

METHOD OF PREPARING CALCIUM CHLORIDE SOLUTION

A discrepancy in the printed directions as to the method of preparing the calcium chloride solution has been noted. Hill's directions (1) state that the calcium chloride solution should contain 378 grams of dry calcium chloride per liter of solution. In a bulletin issued by the Heusser Instrument Manufacturing Company (5) the statement is made that the calcium chloride solution is prepared by adding 378 grams of dry calcium to one liter of water. To determine whether or not the method of preparing the calcium chloride solution had any material influence on the results of the test, two solutions of calcium chloride were prepared according to the above directions. The total volume of the two (finished) solutions was 1000 cc. and 1166 cc. respectively at 20° C. The results of this experiment are

noted in table 6. It will be observed that there was no significant difference in the results when the two calcium chloride solutions were used.

METHOD OF ADDING THE COAGULANT TO THE MILK

Monier and Sommer (2) have recommended that the milk and coagulant be mixed by pouring the milk into the cylinder containing the coagulant. Hill's (1) directions call for adding the coagulant by means of a 10 cc.

TABLE 6

Effect of the method of preparing calcium chloride solution on the results of the Hill curd test

TESTS NUMBER	METHOD OF PREPARING CALCIUM CHLORIDE SOLUTION	AVERAGE CURD TENS IN GRAMS
36	378 Grams of Calcium Chloride Made up to One Liter of Solution	45.88 \pm .37
36	378 Grams of Calcium Chloride Added to One Liter of Distilled Water	47.47 \pm .41

pipette, giving the milk a rotary motion while the coagulant is being added. These two methods of mixing the coagulant and milk were compared in the present study. The results are given in table 7. It will be noted that these results show no significant difference.

The authors have demonstrated to their own satisfaction that this newer method of adding the coagulant is much to be preferred to the procedure

TABLE 7

Effect of the method of adding coagulant on the results of the Hill curd test

TESTS NUMBER	METHOD OF ADDING COAGULANT	AVERAGE CURD TENSION IN GRAMS
36	Coagulant Added to the Milk by Means of a 10 cc. Pipette	38.44 \pm .40
36	Milk Added to the Coagulant	38.72 \pm .39

recommended by Hill. It is fully as accurate, results in a thorough mixing of the milk and coagulant, and has the added advantage of being more rapid. There is every reason in favor of its adoption.

DISCUSSION

The data presented in this paper indicate that results secured with the Hill curd test may be influenced by a number of factors. Even when conditions are as carefully controlled as possible and the test performed by an experienced operator, it is still subject to considerable variation. This being the case, the results of a single determination on a sample of milk is not a reliable index of its curd tension. If a wide discrepancy (greater than 5 grams) occurs when a sample is run in duplicate, the question

naturally arises as to which figure is more nearly correct. To overcome this difficulty and to make certain of a reasonably accurate test the writers have adopted the policy of running all samples in triplicate. By following this procedure two of the determinations will generally agree quite closely.

While the curd test does yield somewhat variable results, it should be borne in mind that in practical use an extremely sensitive test is not required. The test is entirely satisfactory as a method for classifying milk into rough groups on the basis of its curd tension. A method is not required, except for research purposes, which will differentiate between milk samples with only a few grams difference in curd tension.

SUMMARY AND CONCLUSIONS

1. Results secured with a relatively large number of determinations on the same samples of milk indicate that the differences between skilled operators will as a rule be small in the curd test.

2. The temperature at which the test is run was found to be one of the most important factors influencing the results of the curd test.

3. A variation in the time interval between the addition of the coagulant and cutting the curd showed a significant influence on the results of the test. The effect was most pronounced with milk of medium curd tension (55-60 grams) than with samples of lower curd tension.

4. A reduction in the amount of pepsin, pepsin-calcium chloride solution, or calcium chloride solution below that specified in the Hill curd test was found to produce higher results. On the other hand increasing the amount of the coagulant resulted in lower values being obtained.

5. The addition of the milk to the coagulant proved to be as accurate a method of mixing the two as the present recommended procedure. It has the added advantages of being more rapid and easier to perform.

6. While any one of the factors studied may not exert a marked influence on results secured in the curd test, with two or more factors uncontrolled the results would be open to question. It is important that all conditions of the test be controlled as carefully as possible if accurate results are to be secured

LITERATURE CITED

- (1) HILL, R. L. Soft curd milk. Utah Agricultural Experiment Station Bulletin 227, 24 p. 1931.
- (2) MONIER, H. B., and SOMMER, H. H. Soft-curd milk. Abstract of papers presented at the 27th Annual Meeting of the American Dairy Science Association, June, 1932, p. 6. 1932.
- (3) MORRIS, M., and RICHARDSON, G. A. The production and use of soft-curd milk. In *Journal of Pediatrics*, Vol. 3, No. 3, p. 449. 1933.
- (4) LIND, C., and JENSEN, N. The effect of rennet on the contraction of the curd. In *Hansen's Dairy Bulletin*, Vol. 18, Nos. 1-2, p. 5. 1933.
- (5) HEUSSEY INSTRUMENT MANUFACTURING COMPANY. The American Curd-O-Meter. Bulletin 54, p. 2. 1932.

E. COLI IN MASTITIS, WITH ACCOMPANYING CHANGES IN MILK COMPOSITION

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It has been known for a number of years that *E. coli* or some very closely related organism may cause Mastitis. The first indication of this would seem to be the work of Lucet (1) in 1889, who obtained non-gelatin liquefying, gram-negative bacilli from twelve cases of mastitis. These organisms were not fully identified, however, and thus cannot be definitely placed as *E. coli*. One year later Guillebeau (2) found twenty-two cases due to an organism which he named *B. Guillebeau*, but which was later identified as *E. coli* by Jensen (3). Steiger (4) in 1904 gives fourteen cases out of forty-six as due to *E. coli*. Henderson (5) reported *E. coli* in twelve out of fourteen cases, but his results would seem to have little value since examination was made late in the course of infection. Jones (6) reported work done by Savage in which only one case in thirty-one could be attributed to *E. coli*. In the same article Jones gave results of a very carefully controlled experiment, in which two cases were due to *E. coli* out of eighty-one examinations. In 1925 Carpenter (7) in a survey of one hundred and fifty cases discovered *E. coli* in only two. Hardenbergh and Schlotthauer (8) examined the herds on four farms during a period of two and one half years, and out of a total of sixty-eight cases of Mastitis six were caused by *E. coli*. All six occurred on a single farm.

Other authors have described the occurrence of *E. coli* in pathological milk, among others Minnett (9) and Proscholdt (10). In no case, however, could any record be found showing analyses of milk prior to the infection. The case presented here should be of interest since samples were secured shortly before and also during the infection.

History of the Case

A registered Jersey cow, in the experiment station herd, giving eleven pounds of milk per milking suddenly dropped to one pound within a period of twelve hours. An infection seemed very likely in view of the following symptoms: loss of appetite, cessation of rumination, dull and depressed appearance, constipation, body temperature of 107° F., and a hard, hot, painful, swollen condition in the left rear quarter of the udder.

Examination of the milk from the abnormal quarter under the micro-

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scope showed practically a pure culture of rod forms. Since this was rather unusual it seemed wise to study this condition further.

Procedure

Samples of milk were taken from the individual quarters in the following manner: The udder was carefully washed with water, dried, washed with 70% alcohol, the first streams of milk were discarded, and the opening of the test canal again washed with alcohol. Collection of the sample was made in sterile sample bottles, which were held in an ice bath until examination was made (time of holding not exceeding two hours).

The milk was examined under the microscope and an attempt was made to isolate the predominating organism. The method of isolation was to make a serial dilution in litmus milk and plate on standard agar, blood agar and eosine methylene blue agar.

Lactose was determined by the method of Soxhlet and Wein (11), chlorides by Volhard's (12) procedure. Hydrogen-ion concentration was determined potentiometrically using a type K Leed's and Northrup potentiometer with a Baily electrode.

Experimental

Since the milk of this cow, as well as that from a group of others, was being used for chemical analysis a 24 hour composite sample of the milk of October 13, three days before the onset, was available. The next sample was secured immediately following the sudden diminution in milk, while the other samples were taken at intervals during the course of the reported condition.

Microscopical examination of the milk on October 16 showed an abnormally high leucocytic count in all quarters with the exception of the right front. Bacteria were found in only the left rear quarter and were definitely short rod forms. By October 20 the milk of all quarters contained an abnormal number of leucocytes.

During the entire period of examination (one week) bacteria were found in only one quarter; for this reason all bacterial results were based on samples collected from this quarter.

A litmus milk serial dilution showed an acid coagulation out to the 10^{-8} tube. Stains of the series demonstrated the presence of a short rod in all dilutions. A streak plate on eosine methylene blue agar from the last tube in the series yielded a gram-negative bacillus which produced a greenish metallic sheen on this media. Plates of the milk directly from the cow made on standard agar, blood agar, and eosine methylene blue agar showed this organism was present in numbers comparable to those found by the serial dilution in litmus milk.

Several colonies were fished and tested on other media with the following results:

Dextrose—Acid and gas
Lactose—Acid and gas
Clark and Lubs—Red to methyl red
Litmus milk—Acid, slight gas
Indole—Produced
Gelatin—Not liquefied

Pathogenicity tests were made on guinea-pigs using an 18 hour broth culture of one of the typical colonies. The procedure used was as follows: 1 cc. was injected into each of two animals. One was given a subcutaneous injection, while the other received the culture by the intraperitoneal route. In both cases death followed within 16 hours, and the organism was isolated from the liver, kidney, spleen, and heart blood. All indications pointed to death being caused by a toxic condition, since autopsy showed red adrenals and local hemorrhagic areas at the point of injection.

Two other animals were inoculated in the same manner with the milk from the infected quarter. In both cases the animals showed noticeable signs of distress for the two days following the injection, but recovered by the third day.

The results of the chemical analysis are summarized in the following table.

Examination of the table shows that the values obtained for the mixed sample before the onset of the infection compare favorably with values given recently by Rosell (13) for normal milk. The samples taken the first day of infection show abnormally low lactose and abnormally high chloride contents in all quarters; being most pronounced in those samples showing an abnormal leucocyte picture. The specific conductance of the milk very closely parallels the results on chlorides. The latter samples also had a pH approaching that of blood, while the right front quarter gave practically a normal value.

In the samples taken four days later milk secretion had decreased to such an extent that determinations were possible only on chlorides. The chloride content increased in the three quarters which failed to show bacteria, but the value in the infected quarter remained at about the same level. At this time also all samples showed an abnormally high leucocyte count.

Summary and Conclusions

The organisms isolated from an acute case of mastitis proved to be a toxic strain of *E. coli*.

In this case the onset was very sudden, and was noticeable at once by both chemical and bacteriological tests. There was also evidence that even

Analysis of the milk from normal and abnormal periods

DATE	QUARTERS OF UDDERS	PH	LACTOSE PER CENT	CHLORIDE PER CENT	SPECIFIC CONDUCTANCE $\times 10^{-4}$ MHOS AT 25°C	MOLS LACTOSE PER 100 GRAMS MILK	MOLS CHLORIDE PER 100 GRAMS MILK	MOLS LACTOSE PLI'S CHLORIDE PER 100 GRAMS MILK	LEUCOCYTES*
Normal Milk									
10/20/33	All	6.42	4.7	0.057	42.8	13.07×10^{-3}	1.61×10^{-3}	14.68×10^{-3}	
	L.F.	7.42	1.41	0.265	- 94.8	3.82×10^{-3}	7.47×10^{-3}	11.39×10^{-3}	+
	R.F.	6.46	3.93	0.127	58.8	10.92×10^{-3}	3.88×10^{-3}	14.50×10^{-3}	-
10/16/33	L.H.	7.48	Trace	0.317	114.2	-	8.95×10^{-3}	8.95×10^{-3}	+
	R.H.	7.32	2.00	0.249	90.0	5.56×10^{-3}	7.02×10^{-3}	12.58×10^{-3}	+
	L.F.			0.299			8.43×10^{-3}		+
	R.F.			0.268			7.55×10^{-3}		+
10/20/33	L.H.			0.312			8.80×10^{-3}		+
	R.H.			0.280			7.90×10^{-3}		+

* (+) Refers to leucocytes in numbers greater than 5,000,000 per ml.

though the infection remains localized in one quarter abnormal milk may be produced in all four quarters.

The results obtained with tests on pH, lactose and chloride determinations were in the same direction as those found by Roselle in latent chronic mastitis.

Both the chemical and microscopical tests used in this work were satisfactory in the diagnosis of an inflammatory condition of the udder.

REFERENCES

- (1) *Rec. Méd. vét.*, 1889, vi, series 7, 423.
- (2) *Landswirtsch. Jahrb. Schweiz.*, 1890, iv.
- (3) *Ergebn. allg. path. u. path. Anat.*, 1897, iv, 830.
- (4) *Centr. Bakteriolog.*, 1^{re} Abt., Orig., 1904, xxxv, 326, 457, 574.
- (5) *J. Compt. Path. and Therap.*, 1904, xvii, 24.
- (6) *J. Expt. Med.*, 1918, xxviii, 149.
- (7) *J. Am. Vet. Med. Assoc.*, 1925, LXVII, N. S. 20, No. 3, 1.
- (8) *J. Infect. Dis.*, 1927, xl, 667.
- (9) *J. Dairy Res.*, 1930, ii, 84.
- (10) *Archiv. f. Wissenschaftliche und Prakt. Tierheilkunde*, 1928, lviii, 485.
- (11) *Methods of Analysis A. O. A. C.*, 1925, 2nd ed., 261.
- (12) Treadwell and Hall—*Anal. Chem.*, ii, 707.
- (13) *La Mammite Streptococcique de la Vache*. Ministère de l'Agriculture de la Province de Québec (1933).

SOME PHYSICO-CHEMICAL PROPERTIES OF LACTOSE

VI. THE SOLUBILITY OF LACTOSE IN SALT SOLUTIONS; THE ISOLATION OF A COMPOUND OF LACTOSE AND CALCIUM CHLORIDE

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INTRODUCTION

While studying the different types of crystals of lactose, formed under different conditions of crystallization, it was observed that if a considerable quantity of fused calcium chloride was added to a hot concentrated solution of lactose, no crystals appeared on cooling. In successive experiments, the concentrations of lactose and of calcium chloride were increased until finally 15 grams of anhydrous calcium chloride had been added to a boiling solution of 15 grams of alpha hydrate in 10 cc. of water. It was necessary to hold the test tube in boiling water and shake for nearly an hour to get all of the material into solution. At room temperature, this solution was still able to dissolve additional alpha hydrate. This could be demonstrated easily by observing with a microscope single crystals immersed in the solution. Using calcium bromide, a similar solution was prepared which could not be induced to crystallize even at -78°C .

This enormous increase in the solubility of lactose in salt solutions seemed worthy of further investigation. It was hoped that it would help to explain the stability of uncrystallized lactose in ice cream, and in milk powders. In such products, it is dissolved in concentrated salt solutions. This increased solubility may also be a factor in the recovery of sugar from mother liquors in the manufacture of lactose. Also, the increased solubility of lactose in salt solutions might be related to the abnormal rotations observed previously. It was hoped that a study of the effect of salts upon the solubility might explain those abnormal rotations.

It has long been known that other sugars show increased solubility in the presence of salts. This is often of importance in the recovery of sugars from molasses. The increased solubility is probably due to the formation of compounds in the solution.

Compounds of sugars with salts seem to be of two types. Sugars are known to act as weak acids, and unite with bases in alkaline solution. This fact has found commercial application in the Steffans process for the recovery of sucrose. Lactose forms compounds of this type. Dubrunfaut (5) reported that lactose forms compounds with three equivalents of sodium or potassium hydroxide, but with only two equivalents of calcium hydroxide.

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Hönig and Rosenfeld (8) also isolated a sodium lactosate which contained two molecules of water. Bleyer and Schmidt (3) found that the equilibrium rotation was shifted to form more beta lactose at high pHs. Troy and Sharp (21) found that crystallization of lactose was retarded at high pHs. Probably both of these effects are due to the formation of salts by the lactose in solution. Hofmeister (7) found that, in alkaline solutions, one molecule of lactose united with five atoms of copper.

Sugars also form compounds with neutral salts. Lippmann (14, page 549) describes a number of such compounds of glucose with the halides of the alkalis, and compounds of fructose with halides of the alkaline earths, with lead chloride, and with lead nitrate. A number of crystalline compounds of sucrose with the alkali halides are also known (1). Mukhin and Ass (16) found that the velocity of mutarotation of glucose was altered by the presence of salts in solution. They believed that compounds were formed in solution having a greater mutarotation velocity. They had observed the effect of salts upon the mutarotation velocity of glucose as early as 1897 but he did not believe that the effect could be explained by the assumption of compound formation. He pointed out (20, page 451) that the existence of such compounds in the solid state is not proof that they exist in solution. Perhaps the most interesting paper on molecular compounds of sugars with salts is that of Dale (4). He reported the preparation of two compounds of mannose with calcium chloride. One had the formula, beta mannose $\cdot \text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, and showed a normal mutarotation in solution. The other compound, mannose $\cdot \text{CaCl}_2 \cdot 4\text{H}_2\text{O}$, showed an abnormal rotation curve which starts negative but becomes positive within a few minutes and then follows the normal mutarotation curve for alpha mannose. Dale believes that this compound contains some unknown form of mannose which reverts to alpha mannose in solution.

Siegfried and Howwjanz (19) apparently prepared a molecular compound consisting of one molecule of lactose and one molecule of calcium carbonate. Lobry de Bruyn and Franchimont (15) also prepared a compound of lactose with ammonia. However, this seems to be a true amine and not a molecular compound. Pucher and Dehn (18), who studied the solubility of lactose in pyridine, reported that "The solubility curve indicates the probable formation in solution of a molecular compound." However, their data are not very convincing. They report a value for the solubility of lactose in pure water at 1° C., which is nearly double the accepted value. Levy (13) and Levy and Doisy (11, 12) found that lactose combines with borates in solution, and that the formation of such compounds leads to errors in the determination of sugars by reduction.

Peter (17) measured the solubility of lactose in sucrose solutions and found that the solubility at 0° C. was reduced nearly one-half by saturating the lactose solution with sucrose. Leighton and Peter (10) studied the

effect of 39 dyes upon the "super solubility" curve of lactose. Only crystal violet and rosaniline hydrochloride or base had any effect at all, and these had only a slight effect. Hunziker and Nisson (9) measured the solubility of lactose in milk and whey. They reported that the milk colloids had no influence upon the solubility. The solubility of lactose in pure water has been studied by several workers. Gillis (6) gives a critical study of their data together with some original data of his own.

The experiments which are to be reported in this section were made in order to answer several questions. It was known that hydrated lactose was exceedingly soluble in concentrated solutions of calcium chloride. It was desired to prove that this increased solubility was due to the existence of compounds in solution, and not to some other cause, and also, if possible, to isolate the unknown compound, or compounds, and to determine their solubility, and the conditions under which they are stable.

Further, it was known that the equilibrium rotation of lactose was altered by relatively low concentrations of calcium chloride or other salt. It was desired to show that the solubility of lactose was also altered in these dilute solutions.

EXPERIMENTS

The first experiments dealt with the solubility of lactose in dilute salt solutions. The solutions were prepared from Baker's Analyzed calcium nitrate and calcium chloride. They were adjusted to neutrality by means of phenol red. The concentrations were then determined by analysis for calcium. The solutions were saturated with lactose by shaking for six days at a temperature of $32^{\circ}\text{C.} \pm 0.5$. It was not possible to determine the lactose in these solutions by direct polarization because of the influence of the calcium chloride upon the specific rotation. For that reason, a series of solutions containing known amounts of lactose dissolved in the salt solution were prepared. After plotting the observed rotations against the lactose content, it was possible to estimate the solubility of lactose from the observed rotation. As a check upon these measurements, the refractive indices of the solutions were determined, using an Abbe refractometer, and the solubility was estimated by a similar extrapolation.

The results which were obtained are shown in table 1. They are not of a high degree of accuracy, but they do show that there is no great increase in the solubility of lactose at moderate salt concentrations such as might be expected from the results with more concentrated solutions. However, the increase in solubility is unmistakable, and it parallels the shift in specific rotation.

Since the solubility of lactose was increased only slightly in a 4 N solution of calcium chloride, it seemed probable that the great solubility indicated by the earlier experiments in the case of the concentrated salt solu-

TABLE 1
Solubility of lactose in salt solutions at 32° C.

SALT USED	CONCENTRATION OF SALT SOLUTION	GRAMS LACTOSE PER 100 GRAMS WATER	
		By refractometer	By polariscope
None		28.6	28.6
CaCl ₂	5.40%	27.6	27.6
"	10.49%	28.4	29.5
"	19.63%	34.4	33.7
Ca(NO ₃) ₂	1 normal	28.4	29.5
"	2 normal	29.8	29.0
"	4 normal	31.2	33.3

The calcium chloride solutions were approximately 1, 2, and 4 normal but were not adjusted to exactly those values.

tions was due to experimental error. Two possible explanations were considered for the failure of the concentrated solutions to crystallize. They might actually be unsaturated and no crystallization could be expected, or they might be so greatly supersaturated that they had reached a metastable condition. The first theory seemed improbable, especially when the solutions were cooled to $-78^{\circ}\text{C}.$, without crystallization. Therefore, an attempt was made to obtain crystals from a more dilute solution. The first trial succeeded. A hot solution of 20 grams of calcium chloride and 20 grams of alpha hydrate in 30 cc. of water gave well formed crystals on cooling. A microscopic examination indicated that they were neither alpha hydrate nor beta anhydride. One of the very concentrated solutions was then diluted with approximately half its volume of water. After 24 hours, that solution also yielded the same type of crystals.

Preparatory to analysis, the crystals were placed in a Gooch crucible and centrifuged to remove mother liquor. After washing with alcohol, the excess alcohol was removed by placing the material under a vacuum (water pump) while warming gently. The material was then analyzed for lactose by means of the polariscope, for chlorides gravimetrically, and for calcium by the oxalate-permanganate method. The apparent formula was found to be $\text{CaCl}_2 \cdot 0.98 \text{ lactose } 6.94\text{H}_2\text{O}.$

At a later date, two other samples of the crystals were prepared from solutions having quite different initial ratios of lactose to calcium chloride. The crystals were sucked dry in a porous crucible. Then the crucible was placed upon a pad of filter paper in a centrifuge cup. After centrifuging for thirty minutes, the crystals were moistened with alcohol and recentrifuged. Then the remainder of the alcohol was removed by placing the crucible under a high vacuum (using a Cenco Hyvac pump) at room temperature for several hours. Analyses were carried out as before, except

that the lactose was estimated colorimetrically by the method of Bierman and Doan (2). It was necessary to centrifuge the solutions before reading in the colorimeter in order to remove the precipitated calcium salts. The results of these analyses are given in Table 2.

TABLE 2
The analysis of two samples of a compound of lactose and calcium chloride

CONSTITUENTS	SAMPLE 7		SAMPLE 9	
	Per cent	Molecules	Per cent	Molecules
CaCl ₂ (from calcium)	19.33		19.05	
CaCl ₂ (from chloride)	19.43	1.00	19.12	1.00
Lactose (as hydrate)	63.4	1.01	62.9	1.01
Balance (water)	17.5	5.52	18.0	5.82

Since these three analyses were made on samples obtained from solutions of different initial ratios of lactose to calcium chloride, there seems little question that a true compound is formed, at least in the solid state. This compound contains one molecule each of lactose and of calcium chloride. It was not so easy to determine the degree of hydration since it was not known how the crystals could be dried without loss of water of crystallization. The material, as analyzed, was observed to increase in weight rapidly if exposed to the air. Two questions remained to be answered: the exact degree of hydration; and the form of the lactose which was present in the compound. The later problem was taken up first.

When the crystals were dissolved in water, the optical rotation decreased slowly. This increase in rotation indicated the presence of alpha lactose, but other explanations might be possible. The observed mutarotation might have been due either to a slow dissociation of the compound in solution liberating either form of lactose or to the mutarotation of some previously unknown modification of lactose. Dale (4) had found the latter to be true in the case of one of his compounds of mannose and calcium chloride. The simplest explanation of the decrease in rotation was that the compound dissociated almost instantly in solution liberating alpha lactose. This theory was tested in two ways.

A study of the mutarotation velocity should give information regarding the nature of the change which was responsible for the change in optical rotation. The mutarotation reactions should consist of two parts: first, a transformation to one of the known forms of sugar; and second, a subsequent transformation to the equilibrium mixture. The relative velocities of these two reactions would determine the value of the mutarotation constant. If the first reaction were much more rapid than the second, and if the dissociation were complete, then the observed constant would approach

the value for the mutarotation of alpha lactose. If the two reactions were of approximately equal velocities, or if the dissociation were not complete, even though quite rapid, then the observed constant would change in value with time. If the first reaction were slow compared with the second, then the observed constant would be much smaller than the constant for the mutarotation of lactose. In order to distinguish between these three cases, a study was made of the mutarotation of a 12 per cent solution of the lactose-calcium chloride compound at 25° C. A series of observations was made in order to detect any change in the value of the constant with time. These data are shown in Table 3. It seems probable that the minor varia-

TABLE 3
The mutarotation constant of alpha lactose · CaCl₂ · 7H₂O at 25° C.

TIME IN MINUTES	$\kappa_1 + \kappa_2$
10	0.530
15	0.522
20	0.510
25	0.524
30	0.524
90	0.531
180	0.521
Average	0.523

The solution contained 12 grams of compound per 100 cc. The pH was approximately 6.8.

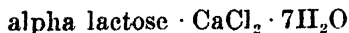
tions are due to experimental error, and that the constant represents the velocity of transformation from alpha lactose to beta.

A second method was used to show that alpha lactose is liberated by the dissociation of the compound in aqueous solution. If the mutarotation were due to a slow transformation of alpha lactose to beta, then the addition of an equivalent amount of beta at the beginning of the experiment would prevent the decrease in rotation. This proved to be true. It was not possible to use the customary value of the beta-alpha ratio because of the shift in equilibrium caused by the calcium chloride. For that reason, a preliminary experiment was performed. The observed rotations were extrapolated back to zero time, and then from the final rotation, it was possible to calculate the beta-alpha ratio, and from that, to calculate the beta equivalent of the alpha lactose present in the compound. The compound, and the equivalent amount of beta lactose were then dissolved simultaneously, and the solution was examined in a polariscope. The first reading that could be obtained was 12.94°. There was no appreciable change in the rotation on standing. After two hours the rotation had increased only to 12.97°. This slight change was considered of no significance, since it was

not greater than the possible error in estimating the beta equivalent. This experiment was considered proof that the compound does dissociate at once liberating alpha lactose, since the chance of any other transformation occurring with precisely the velocity required to prevent mutarotation was very small.

The existence of this compound of salt and alpha lactose is believed to give rise to the anomalous rotation of lactose in calcium chloride solutions. By analogy, it may be assumed that the other cases of abnormal rotation of lactose in salt solutions are due to the formation of similar compounds. In the majority of cases, these seem to contain the alpha form. It is quite possible that many of these compounds can never be isolated in the crystalline form because of unfavorable solubility relations, but the assumption of their existence in solutions seems justified and may prove useful. No serious attempt was made to isolate compounds of lactose with other salts. However, it was noticed that a concentrated solution of lactose and calcium bromide showed a marked mutarotation when diluted, the mutarotation constant being 0.56.¹

The true water content of the compound of lactose and calcium chloride was not yet known. Previous determinations had given values lying between six and seven molecules, and it was believed that the variation was not due to error in analysis, but to improper preparation of the sample. It had been observed previously that the material tended to increase in weight unless protected from the air. Experiments with samples 7 and 9 indicated that when the compound was exposed to the atmosphere, it soon reached a constant weight without any apparent deliquescence. (At the time, the relative humidity was low.) The moisture content of this material was determined by drying under a vacuum with P_2O_5 . During the drying period, the temperature was raised slowly to 105° C. The moisture contents of these samples were found to be 21.70 and 21.77 per cent. The theoretical moisture content of the compound containing seven molecules of water is 21.75 per cent. This was considered to be satisfactory agreement, and the formula



was assigned to the compound.

In order to learn more about the properties of this compound of lactose and calcium chloride, the solubility relations in the system lactose, calcium chloride, and water were studied at 25° C. This is a pseudo quaternary system but under suitable conditions it may be regarded as ternary. A series of solutions of different concentrations of calcium chloride were prepared from Bakers Analyzed chemicals. The solutions were clear, and

¹ Since the preparation of this manuscript, a crystalline compound of lactose and calcium bromide has been isolated. Its formula is apparently similar to that of the chloride compound.

TABLE 4
Solubility relations in the system: lactose, water, and $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$ at 25°C .

SOLUTION	SOLID PHASE	ALPHA HYDRATE	$\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$
1	alpha hydrate	20.65%	0.00%
2	" "	17.6	12.9
3	" "	18.3	24.9
4	" "	20.2	31.6
5	" "	21.7	35.9
6	alpha + compound	21.7	35.4
7	compound	10.0	46.7
8	"	6.1	54.0
9	"	3.75	66.7
10	"	4.57	75.5
11	compound + $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$	8.20	80.5
12	$\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$	0.00	88.5

An analysis of the crystals from solutions 7 and 9 is shown in table 2.

neutral to phenol red. After a trial run to determine the approximate solubility of lactose in these solutions, new solutions of lactose, which contained a small excess of sugar, were prepared by heating. After cooling to 25°C ., each solution was seeded with alpha hydrate, with the compound, and with

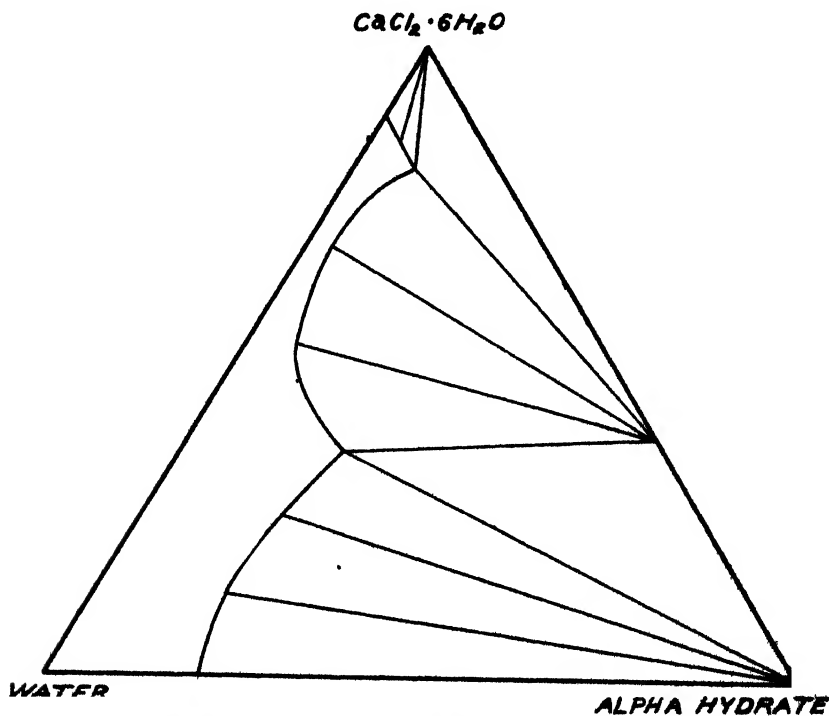


FIG. 1. SOLUBILITY RELATIONS AT 25°C . PER CENT BY WEIGHT, DATA OF TABLE 4

$\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$. The large pyrex test-tubes which contained these solutions were placed in a rocking machine which was suspended in a thermostat. Samples were withdrawn for analysis after three, and after ten days. The crystals were examined with the aid of a microscope in order to determine what solid phase, or phases, were present. The second series of analyses gave values only slightly lower than the first. The data are shown in table 4, and graphically in figure 1. For convenience, the components chosen were water, alpha hydrate, and $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$. The analyses of the solutions were made by a gravimetric determination of chlorides, and by the colorimetric determination (2) of lactose.

SUMMARY

Lactose is more soluble in molar solutions of calcium chloride or of calcium nitrate than in pure water. Increase in the concentration of salt brings further increase in the solubility of lactose. This phenomenon is believed to be related to the anomalous rotations of lactose in salt solutions.

The increased solubility of lactose in calcium chloride solutions is due to the formation of a molecular compound of alpha lactose and calcium chloride in solution. A study of the solubility of lactose in calcium chloride solutions indicated the existence of only one compound at 25°C . This compound was isolated. It has the composition alpha lactose $\cdot \text{CaCl}_2 \cdot 7\text{H}_2\text{O}$.

Hydrated lactose is exceedingly soluble in concentrated solutions of calcium chloride, (or calcium bromide), but these solutions are supersaturated with respect to the compound just mentioned. Such solutions are often exceedingly stable, and may resist all attempts to induce crystallization.

Faith in the reliability of polariscopic data as evidence of the formation of lactose compounds in salt solutions has been strengthened by the fact that lactose, in the solutions tested, exhibited an increased solubility, and furthermore, by the fact that one such compound has been isolated. Such data indicate that many salts may combine with lactose. The existence of such molecular compounds is possibly a factor contributing to the stability of such supersaturated solutions of lactose as are found in ice cream and in milk powders.

BIBLIOGRAPHY

- (1) ALLEN, A. H. Commercial Organic Analysis. 4th edition. Blakiston's, P., Son & Co., Philadelphia.
- (2) BIEMAN, H. R., AND DOAN, F. J. A colorimetric picric acid method for determining lactose. JOURNAL DAIRY SCIENCE 7: 381-92. 1924.
- (3) BLEYER, B., AND SCHMIDT, H. Studien über das Verhalten der wichtigsten Kohlenhydrate in stark saur alkalischer sulfid und bisulfithaltiger Lösung. III. Einwirkung von Alkalien auf der Kohlenhydrate. Biochem. Z. 141: 278-96. 1923.
- (4) DALE, J. K. Relations between rotatory power and structure in the sugar groups. XX. Two isomeric crystalline compounds of d-mannose with calcium chloride. J. Am. Chem. Soc. 51: 2788-95. 1929.

- (5) DUBRUNFAUT. Note sur le sucre de lait. *Compt. rend.* 42: 228-33. 1856.
- (6) GILLIS, J. Solubilité du sucre de lait. *Rec. trav. chim.* 39: 677-8. 1920.
- (7) HOFMEISTER, FRANZ. Beitrage zur Kenntniss der Amidosäuren, p. 28. *Ann.* 189: 6-43. 1877.
- (8) HÖNIG, M., AND ROSENFELD, M. Zur Kenntniss einiger Zuckerarten. *Ber.* 12: 45-7. 1879.
- (9) HUNZIKER, O. F., AND NISSON, B. II. Lactose solubility and lactose crystal formation. I. Lactose solubility. *JOURNAL DAIRY SCIENCE* 9: 517-37. 1926.
- (10) LEIGHTON, A., AND PETER, P. N. Factors influencing the crystallization of lactose. *Proceedings World's Dairy Congress* 1: 477-85. 1924.
- (11) LEVY, MILTON, AND DOISY, EDWARD A. The effect of borate on the oxidation of glucose and other sugars. *J. Biol. Chem.* 77: 733-51. 1928.
- (12) LEVY, MILTON, AND DOISY, EDWARD A. The reaction of borate and sugars. II. The optical activity of sugars in borax solutions and the configuration of mutarotatory isomers. *J. Biol. Chem.* 84: 749-62. 1929.
- (13) LEVY, MILTON. The reaction of borate and sugars. III. The freezing point lowering of sugars in borax solutions. *J. Biol. Chem.* 84: 763-69. 1929.
- (14) LIPPMANN. *Chemie der Zuckerarten*. 3rd edition. Friederich Vieweg und Sohn, Braunschweig. 1904.
- (15) LOBBY DE BRUYN, C. A., AND FRANCHIMONT, A. P. N. Dérivés ammoniacaux cristallisés d'hydrates de carbone. *Rec. trav. chim.* 12: 286-89. 1893.
- (16) MUKHIN, G., AND ASS. T. Action of salts on the mutarotation of glucose. *J. chim. Ukraine* 1: 458-75 (1925) (not seen). *Chem. Zentr.* (1926) 1: 620 (1926). *C. A.* 20: 2775. 1926.
- (17) PETER, PHILIP N. Solubility relationships of lactose-sucrose solutions. I. Lactose-sucrose solubilities at low temperature. *J. Phys. Chem.* 32: 1856-64. 1928.
- (18) PUCHER, GEORGE, AND DEHN, W. M. Solubilities in mixtures of two compounds. *J. Am. Chem. Soc.* 43: 1753-58. 1921.
- (19) SIEGFRIED AND HOWWJANZ. Über die Bindung von Kohlensäure durch Alcohole, Zucker und Oxyssäuren. *Z. physiol. Chem.* 59: 376-404. 1909.
- (20) TREY, HEINRICH. Ein weiter Beitrag zur Biorotation der Glycose. *Z. physik. Chem.* 22: 424-63. 1897.
- (21) TROY, H. C., AND SHARP, PAUL FRANCIS. Alpha and beta lactose in some milk products. *JOURNAL DAIRY SCIENCE* 13: 140-57. 1930.

MILK AND BUTTERFAT YIELDS OF JERSEY COWS AS AFFECTED BY FREQUENCY OF MILKING

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During the five year period from 1929 to 1933 inclusive a total of 14077 Register of Merit records were completed on Jersey cows. Of this number 32.71 per cent of the cows in making their records were milked only twice daily during the entire lactations. There were 33.22 per cent which were milked three times daily for several months and then completed their lactations on twice a day milking. The percentage milked three times daily during their entire lactations was 30.45. The remainder, totalling 3.62 per cent were milked four times daily during a part or all of their lactations.

In years past, many breeders have hesitated to undertake official testing, unless they were able to feed and milk their cows at least three times a day, feeling that on twice a day milking their cows could not possibly make creditable records. This belief is still prevalent to some extent and is one of the reasons frequently offered by breeders for not undertaking Herd Improvement Registry testing. They think that their Herd Test records made on twice a day milking will not be at all comparable to the official Register of Merit or Advanced Registry records of their neighbors' herds made on three time a day milking.

Now that all of the Dairy Cattle Breed Associations have adopted the Herd Improvement Registry, it becomes a very important question as to just what the attitude of these associations shall be regarding the production testing of purebred animals. Should the associations put all of their energy behind Herd Testing alone or should they encourage Herd Testing but also advocate that breeders combine it with semi-official work and continue to test their better cows in the Register of Merit or the Advanced Registry with the advantage of better care and three time a day milking?

Several important questions are involved. First, how much more will a cow produce milked three times a day than if milked only twice daily? Then, does the increase (if any) depend on the relative producing ability of the cow? Is it true that a mediocre cow may very nearly reach the limit of her ability on twice a day milking? Will mass testing of herds on twice a day milking under average farm care really differentiate between cows of average ability and the higher producing cows of the breed? Finally, granting that high records possess worth while commercial and advertising value, from a breed improvement standpoint is it absolutely necessary to

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locate the cows of the breed capable of producing 800 to 1,000 pounds of butterfat in a year's test?

To try to answer, at least partially, some of these questions especially as affecting the policy of the Breed Associations and the attitude of breeders in regard to production testing, an analysis has been made from the Register of Merit records completed by registered Jersey cows. Since 1921, the total number of milkings for all Register of Merit records have been published in the annual record volumes. A check of all these records revealed that since 1921 to December 31st, 1933, there have been just 226 Jersey cows which have completed one record made on strictly twice a day milking during the entire lactation and another record made on strictly three time a day milking during the entire lactation. In all but 19 instances both records on each cow were made under the same ownership. In 184 instances, the record made on twice a day milking was finished first and in the remaining 42 cases, the record made on three time a day milking was completed first. While exact information is not available it is naturally assumed that in most cases at least feeding periods corresponded to milking periods and that cows milked twice daily were also fed twice daily and that cows milked three times daily were fed three times each day.

All of these records were converted to a uniform age basis using the American Jersey Cattle Club age conversion factors. Then the butterfat and milk yields of each record made on twice a day milking were compared with the yields of the record made on three time a day milking and the conversion factor determined both for milk and butterfat. Table 1 is a frequency table of these factors. It will be observed that the greatest frequency falls between 1.00 and 1.29, in fact, 63.27 per cent of all the records fall in this range. There is however considerable variation. For instance in 27 cases, or 11.95 per cent, the record made on three time a day milking was less than the record completed on twice a day milking.

In averaging the factors for converting records made on twice a day milking to a three time a day equivalent, three sets of averages were secured. The first group, including 184 cows on which the twice a day records were made first, showed factors of 1.194 for butterfat and 1.226 for milk yield. The second group of 42 cases in which the three time a day records were made first gave factors of 1.153 and 1.134 for butterfat and milk yield respectively. Totalling the entire group of 226 comparisons results in factors of 1.186 for butterfat and 1.209 for milk yield. In other words, on an average, the cows produced approximately 19 per cent more butterfat and 21 per cent more milk when milked three times daily as compared to twice a day milking. These results compare favorably with those published by Woodward (1) in 1931 but are somewhat lower than the conversion factors reported by Davis and Morgan (2). However, Woodward

TABLE 1
Frequency table showing the distribution of the conversion factors of the 226 comparisons

	.70	.80	.90	1.00	1.10	1.20	1.30	1.40	1.50	1.60	1.70	1.80
	to	to	to	to	to	to	to	to	to	to	to	to
	.79	.89	.99	1.09	1.19	1.29	1.39	1.49	1.59	1.69	1.79	1.89
184 cases in which the (2 time) record was made first												
Number	1	9	11	27	56	33	23	14	5	2	1	2
Percentage	.54	4.89	5.98	14.67	30.44	17.94	12.50	7.61	2.72	1.09	.54	1.09
42 cases in which the (3 time) record was made first												
Number	0	3	3	11	9	7	4	4	0	1	0	0
Percentage	0	7.14	7.14	26.19	21.43	16.69	9.52	9.52	0	2.38	0	0
Totals including all 226 cows												
Number	1	12	14	38	65	40	27	18	5	3	1	2
Percentage	.44	5.31	6.20	16.81	28.76	17.70	11.95	7.97	2.21	1.33	.44	.89

reported a slightly greater increase in butterfat than in milk yield when cows were milked three times daily.

A general belief among dairymen is that good feed and care together with a prolonged lactation period aids materially in developing dairy heifers. Graves (3) has previously shown that the increase in yield of a re-

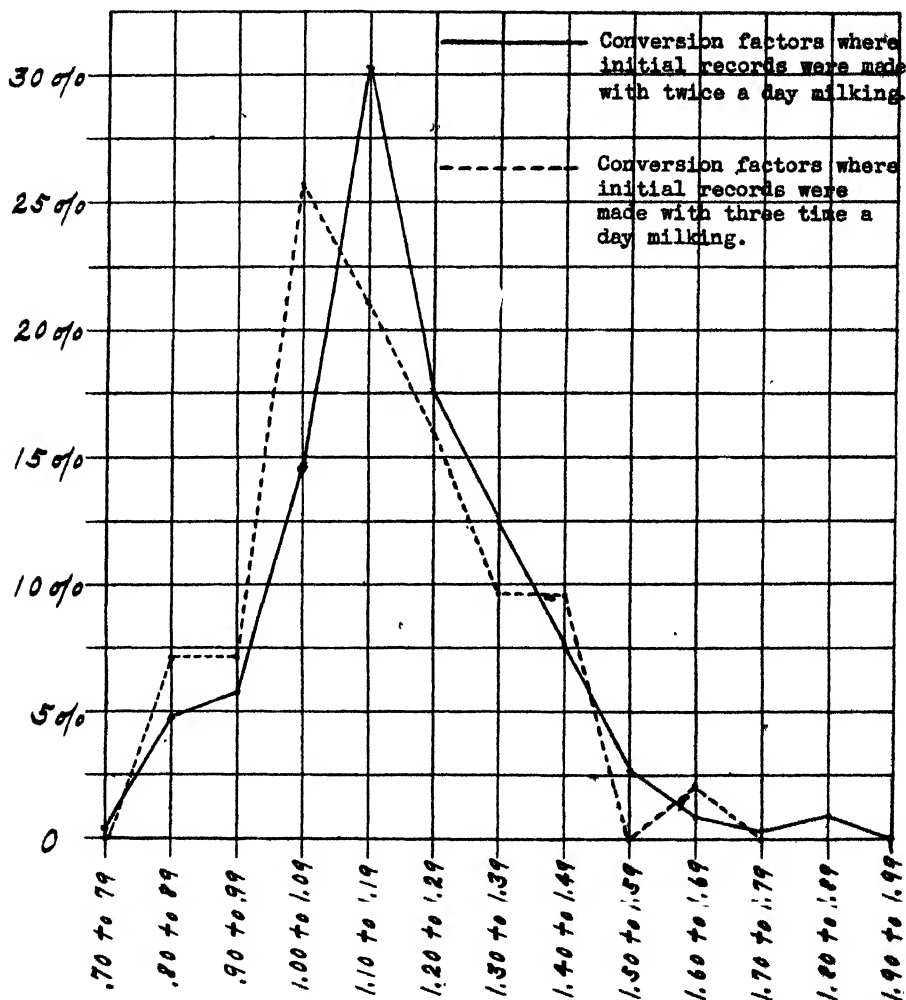


FIG. 1. SHOWING RANGE OF CONVERSION FACTORS WHERE INITIAL RECORDS WERE MADE ON TWICE A DAY MILKING AND WHERE THE INITIAL RECORDS WERE MADE ON THREE TIME A DAY MILKING

entry record compared to the initial record was partly due to increased age and partly to such development which the animal undergoes during the progress of her first official test. He calls this the factor of development and his results show it to be 11.0 per cent for reentry records of Jerseys.

and 12.2 per cent for Guernseys. In his work he did not take into consideration whether the initial records were made on twice a day milking or on three time a day milking.

Figure 1 shows clearly the tendency of the conversion factors to be less when three time a day milking records were made first. This would indicate that when the initial record was made with three time a day milking, the effect on the future production was greater than when the initial record was made with twice a day milking. On a percentage basis this increase in development figures 3.43 per cent for butterfat and 7.50 per cent for milk yield.

The 226 examples were divided into eight groups depending on the records of the cows made on twice a day milking. Conversion factors were determined for each group. The results are summarized in table 2.

TABLE 2
Frequency table showing conversion factors based on producing ability of the cows

PRODUCTION GROUPS BASED ON FAT RECORDS MADE ON TWICE A DAY MILKING	NUMBER OF RECORDS IN EACH GROUP	FACTORS FOR CONVERTING RECORDS MADE ON TWICE A DAY MILKING TO A THREE MILKING PER DAY BASIS
400 to 449	21	1.382
450 to 499	36	1.262
500 to 549	50	1.233
550 to 599	44	1.142
600 to 649	27	1.117
650 to 699	19	1.086
700 to 749	12	1.073
750 and over	17	1.058

These results are quite startling and are in almost direct contrast to popular opinion among many dairymen. However, as the producing ability of the cows increased the gain in production through three time a day milking becomes regularly and constantly less. Swett (4) has demonstrated that the secretion of milk to a considerable extent is a continuous process and Woodward (1) states, "As the udder fills with milk, the pressure exerted by the milk tends to check secretion, and the greater the pressure the more completely is secretion stopped." This will account for the fact that cows yield greater quantities of milk when milked oftener than twice a day. Yet, it seems that according to this reasoning high producing cows ought to show a greater increase than do average producing cows when they are milked three times daily, unless it can be assumed that the high

producing animals have proportionately larger mammary systems so that even on twice a day milking little if any extreme udder pressure is developed. From the results shown in table 2, it is clearly evident that high producing cows can come nearer reaching the maximum of their inherited producing ability on twice a day milking than will animals of average or mediocre ability. These results may be of value in connection with further studies on milk secretion.

To show the expected increase in butterfat yield that might be anticipated from milking three times daily during the entire lactation, table 3 is presented. This table gives hypothetical productions for records made on twice a day milking and shows the expected yields resulting from three time a day milking together with the pounds of butterfat increase.

TABLE 3
*Predicted increases in butterfat production resulting from three
time a day milking*

HYPOTHETICAL YIELDS OF RECORDS MADE ON TWICE A DAY MILKING	CONVERSION FACTORS	EXPECTED YIELD OF RECORDS MADE ON THREE TIME A DAY MILKING	LBS. FAT INCREASE DUE TO THREE TIME A DAY MILKING
425	1.382	587	162
475	1.262	599	124
525	1.233	647	122
575	1.142	657	82
625	1.117	698	73
675	1.086	733	58
725	1.073	778	52
775	1.058	820	45

The 226 records made on twice a day milking were ranked in order of their butterfat yields. These yields were then compared or correlated with the yields which resulted from three time a day milking. This was done to ascertain if the cows would rank the same or nearly the same if milked twice daily as they would if milked three times daily. A fairly close agreement was observed. In fact, the coefficient of correlation found in comparing the two groups was $+ .638 \pm .029$. For a check on these results a search was made through the Second Consolidated Register of Merit volume and a total of 176 cases were found of cows having two records both of which were made on strictly three time a day milking during the entire lactations. These records were all computed to maturity and the cows were ranked in order of the yields of their first records. This ranking was then

compared to the way the same group of cows ranked on the basis of their second records. In this case the correlation coefficient was $+ .642 \pm .031$.

These correlation coefficients are high and are very similar to those of the first comparison. They indicate that a record made on twice a day milking will give almost as good a prediction of what a cow will produce in a given lactation on three time a day milking as does an earlier record made on three time a day milking by the same cow.

SUMMARY

1. Milking Jersey cows three times daily results in an average increase of approximately 19 per cent in butterfat and 21 per cent in milk yield.

2. The use of a single factor for converting all records made with twice a day milking to a three milking per day basis is not recommended.

3. The increase in yield due to milking three times daily, varies greatly with the producing ability of the animal on twice a day milking and is inversely proportional to such ability. High producing cows show a smaller increase in yield when milked three times daily than do cows with a lower inherited producing ability.

4. Milking heifers three times daily on test results in a slightly greater development than when the initial records are made on twice a day milking.

5. Mass testing of cows in the Herd Improvement Registry on twice a day milking will definitely differentiate between average cows and the high producing cows of the breed.

REFERENCES

- (1) WOODWARD, T. E. The production of dairy cows as affected by frequency and regularity of milking and feeding. U. S. D. A. Circ. 180. 1931.
- (2) DAVIS, H. P., AND MORGAN, R. F. The influence of the number of milkings on production of dairy cows. Neb. Agr. Exp. Station Res. Bul. 59. 1931.
- (3) GRAVES, R. R., AND FOHRMAN, M. H. Effect of age and development on butterfat production of Register of Merit Jersey and Advanced Registry Guernsey Cattle. U. S. D. A. Dept. Bul. 1352. 1925.
- (4) SWETT, W. W. Relation of conformation and anatomy of the dairy cow to her milk and butterfat producing capacity. JOUR. DAIRY SCI. 10: 1-14. 1927.

American Dairy Science Association Announcements

NEW OFFICERS

The new officers of the American Dairy Science Association are as follows: President—C. L. Roadhouse, University of California, Davis, California, and Vice-President H. A. Ruehe, of the University of Illinois. The Vice-President of the American Dairy Science Association automatically advances to the Presidency at the end of his term as Vice-President. M. Mortensen of the Iowa State College was elected a Director for a 3 year term to succeed Earl Weaver of Oklahoma. The other two members of the Board of Directors are O. F. Hunziker and L. A. Rogers. The term of office for Roadhouse, Ruehe and Mortensen dates from October 1st. A. C. Dahlberg, New York Agricultural Experiment Station, Geneva, N. Y., continues as Editor of the JOURNAL OF DAIRY SCIENCE, and R. R. Graves, Bureau of Dairy Industry, Washington, D. C., continues as Secretary-Treasurer of the Association.

The officers of the various Sections and Divisions have been corrected on the directory page of the Journal but two Divisions have not yet held their elections.

WESTERN DIVISION MEETING

The Western Division held its annual meeting in the Multnomah Hotel in Portland, Oregon, on October 7, 1934. As in former years, this Division had an interesting program and in the morning there were two sections meeting simultaneously. This Division sponsored the Students' Judging Contest in dairy cattle and dairy products at the Pacific International Livestock Exposition. Of general interest is the following resolution adopted at the business session.

"Whereas the present legal standards and definitions for milk obtaining generally throughout the United States are so high as to discriminate against certain herds of cattle.

"Be it resolved, therefore, that the Western Division of the American Dairy Science Association recommends the establishment of standards or definitions for milk that will not discriminate against the normal milk of any healthy cow."

EASTERN DIVISION MEETING

The Eastern Division held its annual meeting on September 16, 17, and 18, 1934, in the Clinton Hotel at Springfield, Massachusetts. This Division again sponsored the Students' Judging Contest in judging dairy cattle and dairy products at the Eastern States Exposition.

NEXT ANNUAL MEETING

The next annual meeting of the American Dairy Science Association will be June 25, 26, and 27 at the University of Minnesota.

AUTHORS MAY SECURE CUTS FOR ILLUSTRATIONS

Cuts for all graphs and pictures in articles published in the Journal may be secured free by authors who request them from The Science Press Printing Company. The first of each year the old cuts on hand will be destroyed so that requests should be made promptly.

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